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COMMUNICATIONS

MECHANISM OF CORNEAL VASCULARIZATION*

BY

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THE fundamental biological phenomenon of new vessel formation is one of enormous complexity, and has excited the interest of scientific workers for many years as shown by the extensive bibliography of the physiological and pathological aspects of the problem. The normal cornea, having the great advantages of avascularity, transparency, and ready accessibility to biomicroscopical examination, has been widely employed as an experimental tissue in these investigations. There are, however, several special features in the process of corneal vascularization which, as we shall subsequently show, prevent an exact analogy with vessel growth elsewhere, thus rendering the cornea, in fact, a somewhat unsuitable medium for the study of the general question of capillary growth, and necessitating a specific approach to the problem of corneal vascular invasion. This paper, although drawing upon the relevant findings in extra-ocular experimental work, is concerned in the main with the mechanism of vascularization as seen in corneal tissue.

The many theories which have been advanced to explain the initiation of new vessel formation in the cornea may be considered under two main headings:

(1) *That in disease processes a substance is elaborated in the cornea which stimulates the limbal vessels to grow towards the site of its maximum concentration,* i.e., *a process combining growth stimulation and positive chemotaxis.*

From experimental evidence it has been assumed for many years that the general biological phenomenon of directed tissue growth, as seen, for example, in the development of the axons of embryonic neuroblasts, may be controlled by rising and falling gradients of chemical concentration. To this class of cell movement the term " chemotropism " has been applied. In the case of capillary penetration, it is possible that the proliferating vascular buds move from a zone where some chemical substance is in low concentration, towards a region of traumatized or necrotic tissue containing this substance in high concentration.

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The release of growth-stimulating substances from tissue cells has been extensively investigated. Thus Fischer (1930) demonstrated the promotion of growth which resulted from the infliction of wounds upon tissue cultures, and Cameron (1935) showed that extracts of inflamed tissue were similarly effective. Such substances, which are particularly active in embryonic tissue, have been demonstrated in extracts of leukocytes and named " trephones " by Carrel (1922). In repair processes Menkin (1941) holds the view that several proliferative factors may be involved; indeed it is known that proteoses (Carrel and Baker, 1926; Carrel and Ebeling, 1928), peptones (Kiaer, 1928), nucleoprotein fractions (Fischer, 1939), glutathione and haemoglobin (Baker, 1929), and the sulphydril group in general (Hammett, 1929) may all be effective stimuli in certain circumstances. In plants it has been shown that growth-stimulating " wound hormones " are liberated from injured cells (Haberlandt, 1922), and more recently one of the substances has been isolated in pure form and named traumatic acid (English, Bonner, and Haagen-Smit, 1939).

The application of this principle to the problem of corneal vascularization has been particularly emphasized by Campbell and Michaelson (1949), who suggested from their studies on standardized burns in rabbits' corneae that corneal new vessel formation involves a factor released at the site of the lesion whence it diffuses to stimulate and direct new vessel growth from the limbal plexus.

Subsequently Campbell and Ferguson (1950) showed that corneal vascularization occurs more readily in scorbutic than in normal guinea-pigs; they believed this indicated an inability of corneal tissue deficient in ascorbic acid to meet the increased metabolic demands of repair; this led to an accumulation of metabolites which might constitute the factor postulated by Campbell and Michaelson (1949).

Many consider that histamine may be the growth-stimulating factor and its possible role has been fully discussed by Offret and Chauvet (1950). Haessler (1927) suggested that the stimulus came from toxins in combination with blood constituents, and Julianelle and Lamb (1934) have demonstrated that the intracorneal injection of egg albumen and bacterial nucleoproteins can give rise to corneal cloudiness followed by vascularization. If such a diffusing vascularizing substance exists, it is perhaps, surprising that it never extends beyond the limbus to stimulate proliferation in the immediately adjacent scleral vessels. Nor does the theory of such a factor assist in the conversely related problem of why the cornea is normally avascular.

Local anoxia or the sequential accumulation of acid metabolites might be the operative stimulus. In the cornea, Bessey and Wolbach (1939) and Johnson and Eckardt (1940) attributed the marked ingrowth of capillaries which follows riboflavine deficiency to a break-down in the corneal oxidative system. Swindle (1938) believed that vessel ingrowth is attributable to an increase in the concentration of hydrogen ions. Indeed, that oxygen-lack

or an excess of carbon dioxide in the tissues is a potent factor in the production of neovascularization generally, is supported by a considerable amount of experimental evidence.

Thus the presence of such influences is implicit in the important observations of Michaelson (1948) upon the development and anatomy of the normal retinal vessels; his preparations demonstrated a capillary-free zone around the retinal arteries, and he found that the formation of retinal capillaries in the developing embryo is pre-eminently a formation of the retinal veins and that if a vein and artery are close to each other, growth takes place predominantly from the side of the vein remote from the neighbouring artery. Experiments which logically follow from these findings have been carried out by Campbell (1951) who subjected litters of rats to a low oxygen environment and found that the capillary-free zone around the retinal arteries was significantly narrowed, thus further tending to show that oxygen tension is closely related to capillary growth.

Anomalous vascular growth has been produced by Byerly (1926), in developing chick embryos asphyxiated by immersion of the egg in water-glass, and Windle, Becker, and Weil (1944), in studying alterations in the brain structure of guinea-pigs which followed asphyxiation at birth, found vascular proliferations in regions where neurons had been destroyed by anoxia. Champy and Louvel (1938) have induced new vessel formation in the frog's foot by immersing it in carbon dioxide, and in the frog's and guinea-pig's mesentery following intraperitoneal injection of the same gas; these authors concluded that carbon dioxide is not only a vaso-dilating agent but also a vaso-formative one.

In conditions in which ischaemia arises as a result of vascular occlusion (such as Eales's disease, occlusion of the central retinal vein, and diabetic retinopathy), anoxia has been held responsible for the new vessel formation which frequently follows. Anoxia is also said to play a part in the angiomatosis of Lindau's disease (Ingalls, 1948) and in the neovascularization which is a characteristic feature of retrolental fibroplasia (Szewczyk, 1952; Ingalls and others, 1952; Crosse and Evans, 1952).

It will thus be seen that the theory of anoxia as a stimulus to new vessel formation demands serious attention and it will be referred to again in our subsequent discussion. It should be borne in mind, however, that even if tissue anoxia were proved to be a stimulating factor in new vessel formation it may, nevertheless, operate indirectly through the release of growth promoting factors.

(2) *That the limbal vessels are normally prevented from entering the cornea either through its chemical content or through growth-inhibiting substances or through the compactness of its tissue. Vascularization would then arise through destruction of the antagonizing substance or through a reduction in corneal compactness.*

The theory postulating the inhibition of new vessel formation by a factor present in the corneal tissue was first advanced by Meyer and Chaffee (1940

a, b) who isolated a sulphate ester of hyaluronic acid from the substantia propria. By analogy with hyaline cartilage, which contains a very similar carbohydrate (chondroitin sulphuric acid), and like the cornea is also avascular, they advanced the hypothesis that the polysaccharide was intimately connected with corneal transparency and the absence of blood vessels. They showed that these characteristics were abolished by hyaluronidase and suggested that various pathological lesions may produce an increase in such enzyme activity and so lead to corneal vascularization. It was in accordance with this concept that Jones and Meyer (1950) considered that the inhibitory effect of cortisone on corneal vascularization might be due to its ability to prevent such a break-down of corneal mucopolysaccharide.

Considerable doubt, however, as to the importance of the role played by corneal mucopoly saccharide has been cast by the work of Wislocki and others (1947) who demonstrated that hyaluronidase does not abolish the metachromatic staining reaction of the cornea, of Woodin (1950), who found that hyaluronidase is inactive as a spreading factor in the cornea, and of Wise (1943), who showed that hyaluronic acid ester was not diminished in quantity in vascularizing corneae.

Although growth inhibiting substances have been demonstrated in normal serum in concentrations increasing with age, and in tumours and liver extracts (Heaton, 1926; Drew, 1927; Brues, Subbarow, Jackson, and Aub, 1940), no inhibiting factors have as yet been demonstrated in corneal extracts.

The thesis that the degree of compactness of the corneal tissue is the determining factor in the initiation of neovasculogenesis was originated by Cogan (1949 a). He rightly pointed out that tissues such as cartilage and finger nails cannot be vascularized because they have no invadable intercellular substance and he believes that these conditions are equally applicable to the cornea. In a series of experiments he produced small corneal lesions by burning with a cautery and by injection with hydrochloric acid or sodium hydroxide and he has observed a regular sequence of events leading to new vessel formation. Engorgement of the limbal vessels was followed by the formation of saccular aneurysms which burst, and new vessels then proliferated into the resulting haemorrhage between the corneal lamellae. The one event which constantly preceded these vascular changes was swelling of the corneal stroma, and he therefore concluded that these vascular developments were attributable to a reduction in the compactness of the corneal tissue.*

Cogan's hypothesis is attractive in that it explains with nicety why vessels are prevented from entering the healthy cornea and how it becomes possible for them to invade it in disease. A point against the argument, however, is that new vessel formation must necessarily involve a proliferation of endothelial cells and although the stimulus for cellular regeneration is unknown, the available evidence is against its being dependent upon alterations in tension; indeed, there has been a general tendency to abandon Ribbert's tissue-tension theory.

*The terms "corneal swelling" and "reduction of tissue compactness" are used synonymously in this paper and in that of Cogan (1949a) because they cannot be distinguished by simple observation. It is theoretically possible, however, that corneal swelling may occur without opening of the intercellular spaces, *e.g.*, from intracellular fluid inhibition; the term "reduction in tissue compactness" implies a stromal separation due to increased extra-cellular fluid and is the more accurate term when referring to a removal of obstruction to ingrowing vessels.

As we have already indicated, it is more probable that the proliferation is a response either to metabolic demands or to growth-promoting substances liberated by injured cells (Davidson, 1943). It would seem, therefore, that some factor in addition to corneal swelling is likely to be involved in the vascularizing process.

It may thus be concluded that despite the attention the subject has attracted, the mechanism of corneal vascularization remains in doubt, and that none of the many theories advanced appears to merit complete acceptance. In an endeavour to clarify the present position, we have carried out further experiments on the problem, repeating some of those of other workers, particularly those of Cogan, and devising methods of our own. The findings are discussed in relation to the above concepts, and an attempt is made to assess their bearing upon clinical appearances and upon the therapeutic action of cortisone.

Experiments

The experiments were designed to investigate the two most important questions in corneal vascularization, namely, the likely roles that may be played by a vascularizing substance and alterations in tissue compactness. Although the two problems overlap to a considerable extent, it will be convenient to consider them under two headings.

(1) To investigate the presence of a vascularizing substance.—The methods employed were those of injecting saline extracts and grafting discs from vascularizing corneae into the corneae of normal eyes. Control experiments were carried out with extracts and grafts from normal corneae; the effects of needle puncture and saline injection were separately controlled. Rabbits anaesthetized with nembutal were used throughout the experiments.

Preparation of Extracts.—Dilute extracts were first employed to avoid the complicating factor of viscous solutions; a more concentrated extract was used later.

(*a*) *Dilute Extracts.*—The control and test extracts were prepared respectively by grinding six normal corneae and six vascularizing corneae in physiological saline in a pestle and mortar. The resulting solution was centrifugalised and the supernatant fluid freeze-dried; the deposit was then weighed and made up to a 5 per cent. solution. (Vascularization was induced by Langham's alloxan technique reported by Ashton, Cook, and Langham, 1951; none of the vessels was included in the corneal discs which were taken for extraction on the 7th to 10th day, when marginal vascularization was established).

(*b*) *Concentrated Extract.*—Thirteen control and eight vascularizing corneae were treated as above and reconstituted to a fully saturated solution (30–40 per cent.).

EXPERIMENT (i).—A series of four animals received an intracorneal injection of 0.02 ml. physiological saline 4 mm. from the limbus in the left eye, and a sterile needle track was made in the right eye 4 mm. from the limbus.

Result.—There was no vascularization in either eye in any of the four animals.

EXPERIMENT (ii).—A series of six rabbits received an intracorneal injection of 0.02 ml. dilute corneal extract 4 mm. from the limbus. The right eye was used for the test extract and the left for the control.

Result.—In three animals both corneae became vascularized, but the ingrowth of vessels in the control eyes was less marked and appeared later than in the test eyes. In one animal both corneae became vascularized but the control eye vascularized earlier than the test eye. In one animal only the test eye vascularized, the control eye remaining normal. In the sixth animal neither eye vascularized. Corneal vascularization was thus inconstant but could

be induced by the extracts of both normal and vascularized cornea (Figs 1 and 2).

FIG. 1.—Experiment (ii). Control rabbit, 6 days after intracorneal injection of control corneal extract (5 per cent. solution). Note deep marginal vascularization extending into area of corneal haze.

FIG. 2.—Experiment (ii). Test rabbit, 6 days after intracorneal injection of extract of vascularized corneae (5 per cent. solution). Note well-marked marginal vascularization of cornea.

EXPERIMENT (iii).—The experiment was exactly similar to that described above except that concentrated extract was used.

Result.—In two animals both corneae vascularized. In one animal only the control cornea vascularized. In one animal only the test cornea vascularized. No difference was, therefore, demonstrated between the test and control experiments.

EXPERIMENT (iv).—In a further series of six animals, 0.02 ml. concentrated corneal extract was injected into the centre of the cornea. The right eye was used for the test extract and the left for the control.

Result.—In both eyes there was a dense central corneal haze but in no case did this extend to the limbus. No vascularization occurred in either test or control eyes.

EXPERIMENT (v).—A series of four rabbits received a central full-thickness corneal graft. In two the grafts consisted of normal corneae, while in the other two the grafts were taken from corneae in which vascularization had been induced by the intracameral injection of alloxan (Figs 3 and 4).

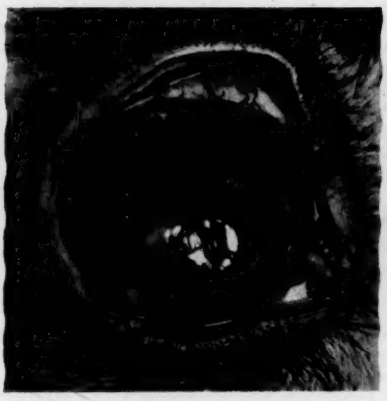

FIG. 3.—Experiment (v). Control rabbit, showing extensive corneal vascularization 21 days after insertion of control graft.

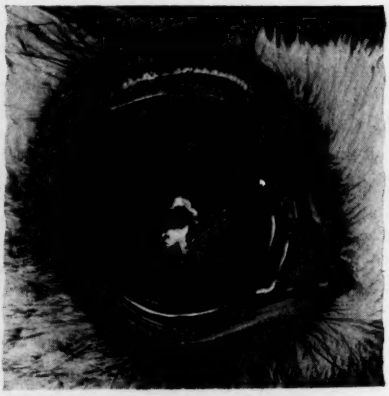

FIG. 4.—Experiment (v).—Test rabbit, showing corneal vascularization 21 days after insertion of graft from a vascularized cornea.

Result.—Corneal vascularization occurred in all four animals.

(2) To evaluate the role played by alterations in tissue compactness.—In these experiments attempts were made to decrease the tissue compactness by opening up the lamellae with a fine flattened probe, by injecting nitrogen, saline, and serum, and by inducing corneal oedema through traumatising the corneal endothelium.

EXPERIMENT (vi).—In two rabbits a plane of cleavage was made through the middle thickness of the cornea by means of a fine flattened probe. The apex of the triangle was 5 mm. from the limbus and the base extended along 15 mm. of the corneo-scleral junction.

Result.—In one eye a 2 mm. zone of vascularization developed after 3 days and by the 6th day it had extended to 3 mm. with cloudiness and swelling of the adjacent cornea. The cloudiness disappeared slowly, and after 10 days the vascularization had disappeared. The plane of cleavage was then re-opened with the probe and well-marked vascularization recurred with marked surrounding corneal swelling, both of which had disappeared by the 12th day.

In the second eye a 2 mm. zone of vascularization appeared by the 3rd day, but by the 6th day there was no corneal oedema and the vascularization had almost disappeared. The plane of cleavage was then re-opened and a well-marked vascularization re-appeared in 3 days, but again both corneal oedema and vascularization had disappeared by the 6th day (Fig. 5).

FIG. 5.—Experiment (vi) Marginal corneal vascularization 3 days after producing plane of cleavage with fine probe.

EXPERIMENT (vii).—Nitrogen was injected into the cornea, towards and 5 mm. from the limbus. The corneal swelling had disappeared within 24 hrs and no vascularization occurred. Two weeks later a further nitrogen injection was made *via* the original site of injection and into the same area.

Result.—In one eye the needle entered the anterior chamber and in 3 days a 2 mm. zone of vascularization occurred. No vascularization developed in the second eye in which the anterior chamber was not entered.

EXPERIMENT (viii).—In three rabbits physiological saline was injected into the cornea.

Result.—The bleb had almost disappeared in 24 hours and no vascularization ensued.

EXPERIMENT (ix).—A daily intracorneal injection of 0.15 ml. physiological saline was given in two eyes. The injection was made approximately 8 mm. from the corneo-scleral junction, and the area of corneal turgescence extended to the limbus on each occasion.

Result.—The corneal opacity was only transient in nature, transparency being regained within 12 hours. After the fifth injection, however, an incipient vascular invasion of the corneal stroma was apparent and after the sixth injection a well-marked intracorneal vascular ingrowth could be seen.

EXPERIMENT (x).—In two eyes 0.02 ml. rabbit serum was injected into the cornea towards and 5 mm. from the limbus.

Result.—The bleb had almost disappeared within 24 hours and no vascularization occurred in either eye. A further injection of serum was then given, at the same site and in the same direction. In one eye a faint corneal haze persisted between the limbus and the needle puncture and two small vascular twigs, 1 mm. and 2 mm. in length, appeared by the 3rd day, and persisted for one week. In the other eye no haze persisted and no vascularization developed.

EXPERIMENT (xi).—Two non-penetrating needle tracks were made into one cornea, one entering 3 mm. from the limbus and the other 5 mm. from the limbus.

Result.—No vascularization occurred in either site.

EXPERIMENT (xii).—Two needle tracks penetrating into the anterior chamber were made, one at 10 o'clock 5 mm. from the limbus and the other at 2 o'clock 3 mm. from the limbus.

Result.—In 3 days one small vessel 1 mm. in length had invaded the cornea opposite the 2 o'clock perforation, and in 6 days two small vessels 2 mm. in length were present. There was, however, no vascularization opposite the needle track 5 mm. from the limbus, the intervening corneal stroma being clear.

EXPERIMENT (xiii).—In three eyes the endothelium was abraded by means of a metallic foreign body previously introduced into the anterior chamber, according to the technique of Cogan (1949b).

Result.—In one eye the abraded area was immediately adjacent to the limbus. A localized area of faint to moderate central haze resulted, but it did not extend to the limbus and no vascularization ensued (Fig. 6). In a second eye the abraded area was 4 mm. from the limbus; again a localized central area of opacity developed, which did not reach the limbus, and no vascularization ensued. In the third eye the central endothelium was abraded, and the result was the same. In all three cases localized corneal vascularization occurred in the region of the original keratome incision.

EXPERIMENT (xiv).—The endothelium and Descemet's membrane in three eyes were removed with a blunt curette from a small area 5 mm. from the limbus.

Result.—In 3 days there was a diffuse corneal haze extending up to the limbus, and a dense 2-mm. zone of corneal vascularization was present. By the 6th day the haze was absorbing rapidly, but the vascularization was 3 mm. in area and increased to 4.5 mm. by the 11th day (Fig. 7).

EXPERIMENT (xv).—The endothelium was removed from a similar area in three eyes with a bent wire covered with polythene tubing.

Result.—In all three eyes a localized area of faint to moderate corneal haze followed; this did not extend to the limbus and no vascularization ensued.

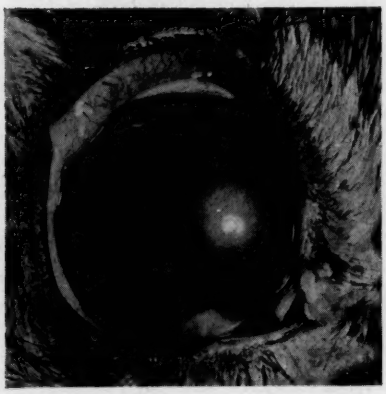

FIG. 6.—Experiment (xiii). Residual central corneal opacity resulting from abrasion of small area of endothelium immediately adjacent to upper limbus.

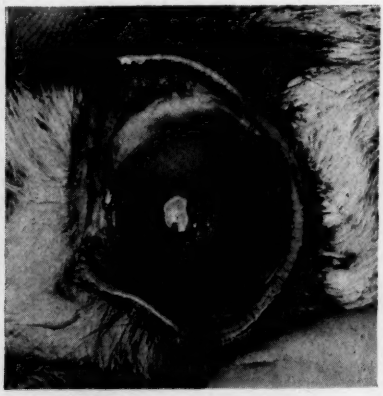

FIG. 7.—Experiment (xiv). Marked corneal haze and zone of peripheral vascularization resulting from removal of small circular area of endothelium and Descemet's membrane 5 mm. from limbus (4th day). Although the corneal epithelium was not damaged, a marked pigment slide has occurred.

Discussion

So far as the production of evidence in favour of a vascularizing substance is concerned, it will be seen that the above experiments failed to demonstrate

any significant difference between normal and vascularized corneal extracts, or between normal corneal grafts and vascularized corneal grafts. Thus normal corneal extracts (Figs 1 and 2) or grafts (Figs 3 and 4) were equally successful in stimulating new vessel formation, as were the extracts and grafts from the vascularizing corneae. The great disadvantage of the experimental technique, however, is that tissue-trauma, which has been held responsible for the release of a vascularizing factor, was involved in the preparation of both control and test extracts and in the control and test grafting experiments. For this reason the grafting experiments could only have been of value if a difference was to have been demonstrated between the test and control grafts. The fact that we obtained vascularization in all grafts is completely inconclusive, but the fact that both the normal and the vascularized corneal extracts failed to produce vascularization when injected into the centre of the cornea, is evidence against the existence of a diffusible vascularizing factor extractable by the methods employed. Nor was there evidence of a directional influence towards the lesion (Fig. 8), the vascularization occurring principally in areas of greatest swelling, even when these were not adjacent to the site of injury; we have also frequently observed new vessels growing beyond the exciting lesion, at which site a diffusible factor might be expected to be at its maximum concentration. There is, of course, a directional influence towards the centre of the cornea. Although we have neither succeeded in devising experiments sufficiently delicate or uncomplicated to prove or disprove the existence of a vascularizing factor, nor excluded the possibility of such a factor being masked by growth-inhibiting substances, we have, nevertheless, found no evidence to support the concept that vascularization occurs in response to the release of a specific "vascularizing substance" in the diseased cornea.

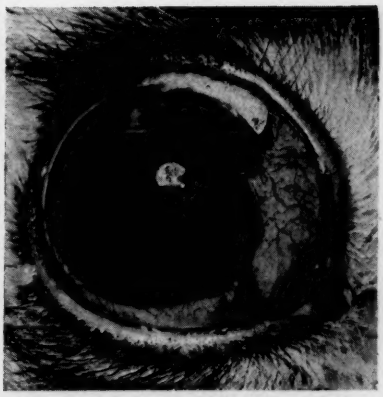

FIG. 8.—Marginal vascularization of cornea induced by corneal laceration re-opened every 3rd day over a period of 17 days. Note that vascularization shows no evidence of directional influence towards the lesion.

On the other hand, the second group of experiments indicates that a reduction in the compactness of the corneal tissue, whether produced mechanically or by the induction of corneal oedema, may give rise to vascularization, providing the potential pathways resulting from the stromal separation communicate with the limbal vessels and are maintained for a sufficient period of time. In no case in either group of experiments was vascularization produced which was not preceded by a zone of corneal swelling extending up to the limbus. Rapidly diffusible substances such as

saline produced no vascularization unless the cornea was repeatedly injected, whereas the viscous and slowly absorbable corneal extract was followed in a high proportion of cases by a well-marked ingrowth of new vessels, provided that the associated corneal swelling itself reached the limbal region.

Our experiments involving the induction of corneal oedema should ideally be considered in relation to normal function. Unfortunately the physiological mechanism whereby the cornea retains its transparency and compactness has been a subject of much controversy for many years, and, although the factors involved have been very thoroughly investigated (Kinsey and Cogan, 1942; Pirie, Schmidt, and Waters, 1948; Davson, 1949; Potts and Johnson, 1950; Maurice, 1951), their mode of operation is as yet unknown. It is certain, however, that the transparency of the cornea is closely connected with its water content (Fischer, 1933; Kinsey and Cogan, 1942), which in turn may be determined by the osmotic forces operating between it and the surrounding fluids. The cornea normally contains 76 per cent. of water, and an addition of 10 per cent. causes it to swell and turn cloudy (Fischer, 1927). Kinsey and Cogan (1942) suggested that the cornea was maintained in a deturgescent and compact state by the hypertonicity of the tears and aqueous fluid, but Davson (1949) and Maurice (1951) thought this theory required modification, and advanced reasons for supposing that the fluid is extruded out of the cornea by some active process. In any event it is clear that, to maintain respiration and control of fluid transference, there must be no impairment of the continuity or functional integrity of the corneal epithelium and endothelium (Rones, 1940). Maurice and Giardini (1951) have shown that after removal of the corneal epithelium the corneal stroma swells to double its previous thickness, whereas the removal of the endothelium is followed by a much greater degree of stromal swelling; this swelling is still further enhanced when Descemet's membrane is ruptured, in which case it may be some six times greater than that produced by epithelial removal alone.

That these facts have a close bearing upon vascularization is shown in our experiments, and in those of Cogan, involving the removal of corneal endothelium.

It was found that the degree of trauma to the endothelium, the extent of the resulting oedema, and the incidence of vascularization were directly related. Thus, when an area of endothelium was completely removed and Descemet's membrane was ruptured, a diffuse corneal thickening and opacification ensued which was followed by a profuse ingrowth of new vessels. When, however, the endothelium alone was abraded, a faint diffuse haze with slight corneal swelling followed and there was no accompanying vascularization. This latter failure to vascularize is attributable, at least in part, to peripheral clearing of the cornea (Fig. 6); a point to be immediately discussed.

The role played by the limbal plexus in the control of corneal hydration has received considerably less attention than those of the epithelium and

endothelium. It has been shown, however, that destruction of the limbal vessels does not give rise to swelling or opacity in the cornea (Gruber, 1894), from which it follows that the factors operating through the epithelium and endothelium are themselves sufficient to maintain normal deturgescence. When, however, corneal swelling arises as a result of minimal endothelial damage, and gives rise to a faint corneal haze, the part played by the limbal vasculature may become apparent. Thus, in our experiments in which this technique was employed, it was noted that the corneal haze and swelling cleared rapidly at the periphery and more slowly centrally, irrespective of the site of endothelial injury. Throughout the endothelial abrasion experiments this central opacity was consistently present.

It would appear, therefore, that in cases of slight endothelial damage the normal fluid exchange between the limbal vessels and the peripheral cornea is sufficient to cope rapidly with excess fluid in this part of the cornea but is insufficient to deal with a central swelling; the latter disappears only after a considerably longer period, and that only after the endothelium has regenerated.

Thus far our investigations fully substantiate Cogan's conclusion that a reduction in the tissue-compactness of the cornea in the region of the pre-existing vessels is an essential precursor of the process of corneal vascularization (Cogan, 1949a). On general principles, however, it is difficult to accept his thesis that new vessel formation in the cornea is solely initiated by a loosening of the tissue spaces at the limbal margin, for this would confer upon the limbal vessels a property which is not apparently possessed by the general vascular system. As we are aware, there is no evidence that a reduction of tissue compactness by chronic oedema, even of long duration, has any influence upon new vessel formation outside the cornea. In the retina, for instance, new vessel growth is certainly not necessarily so associated. The compactness of the corneal tissue, like the compactness of cartilage, may be regarded merely as an obstruction to invading vessels; the fact that a reduction in this density allows newly formed vessels to enter the tissue does not necessarily imply a stimulus to neovascularization. As has been indicated earlier in this paper, new vessel formation is more likely to arise as a response to some growth-promoting substance resulting from injury to the tissues of which anoxia may be an example; it is even possible that anoxia may directly stimulate growth.

Our experimental findings do not entitle us to draw conclusions beyond this point, but since our experiments and those of Cogan have failed to demonstrate any extractable or diffusible vascularizing factor, it may be helpful in planning future research work, to speculate upon the presence of other stimulating influences, which can be accepted as being generally applicable to the vascular system as a whole.

Since corneal vascularization can be produced by simple separation of the corneal lamellae (*e.g.*, with saline) it would appear likely that the vascular-

izing stimulus, the existence of which seems both logical and necessary, is constantly present in an active form in the normal cornea. It is, therefore, instructive to refer to the recent researches of Langham (1952b) who has shown that the cornea in the living animal does not utilize oxygen to its maximal capacity; in experiments *in vitro*, however, this deficit was made good owing to the more ready availability of the atmospheric oxygen. In other words, the cornea *in vivo* is constantly and normally in a state analagous to that pertaining in anoxia, which, as we have already shown, is strongly supported experimentally as a potent stimulus to vascularization throughout the body. If this condition of " suboxidation " can give rise to neovascularization, then such a stimulus permanently resides in normal corneal tissue.*

This finding of Langham's would, therefore, complete a possible hypothesis for the mechanism of corneal vascularization, namely: vascularization is normally prevented by the compactness of the tissue and when this is decreased, vascularization will readily occur, in response to a stimulus which is constantly present in the normal cornea, provided the potential pathways resulting from the stromal separation are adjacent to the limbus and maintained open for a sufficiently long time.

The vast majority of the experimental findings in the literature can be satisfactorily explained on the basis of the above mechanism, but we shall turn now to a consideration of those reports which would appear to be opposed to it. Although the idea of " suboxidation " as the vasoformative factor in the cornea has many points in common with the concept of Campbell and Michaelson (1949), it differs in some respects. It does not embrace their idea of a substance released in injured tissue and diffusing to the limbus, but their experimental results may be explained otherwise than by postulating such a factor. The small standard burns which they produced in the cornea would have given rise, through epithelial damage and through inflammation, to a local corneal oedema, distributed in a circular fashion around the lesion. As Cogan (1949 a) and our own experiments have shown, vascularization would then only occur providing the oedematous tissue was in contact with the limbal vessels, and, in the case of a small lesion, this in turn would be dependent upon its distance from the limbus. Such a process would explain both their findings of a " critical distance " and the triangular segmental zone of vascularization. Corneal oedema, therefore, together with the metabolic conditions prevailing in the normal corneal tissue, provides an equally satisfactory explanation of their results.

A much more serious challenge to the above hypothesis is that of Bessey and Wolbach (1939), who found that the vascular ingrowth induced by riboflavin deficiency was unaccompanied by any change in corneal transparency (and, therefore, presumably in corneal thickness), until many days and often

*The term suboxidation used in this context is not strictly accurate in that it connotes a pathological state in which the oxygen supply is deficient for the tissues normal requirements, whereas the cornea normally consumes less oxygen than it is capable of utilizing in abnormal circumstances. Nevertheless, the normal corneal metabolism is comparable to that found in anoxic tissues, and the word " suboxidation ", in the absence of a better term, is used in this sense throughout the discussion.

several weeks after the invading vessels became visible. Furthermore, neither the corneal endothelium, Descemet's membrane, nor the corneal epithelium showed any demonstrable changes until late in the deficiency. Their thorough experimental technique provides no obvious escape to the conclusion that the vessels were able to invade a compact corneal tissue—a faculty which is denied by the bulk of experimental work on corneal vascularization. There may, however, be many ways in which corneal vascularization can arise, the mechanism we have suggested being but one, and it may be that special unknown factors come into play in riboflavin deficiency. Alternatively, it is possible that some diminution in compactness may occur without a readily recognizable change in transparency. Indeed, if vital factors are involved in the maintenance of corneal deturgescence, as suggested by Davson (1949) and Maurice (1951), one would expect them to be affected in a deficiency which undermines the very foundations of cellular respiration. It would be interesting to repeat the experiments of Bessey and Wolbach (1939) with accurate measurements of corneal thickness; their suggestion that the vascular obliteration which follows correction of the riboflavin deficiency, is due to a compression between the corneal lamellae, certainly implies a reversal of corneal swelling. Furthermore, other workers (Totter and Day, 1942) have stated that in riboflavin deficiency vascularization occurs only after a diffuse corneal opacity becomes apparent.

There is also some difficulty in understanding the mechanism of corneal vascularization in tryptophane deficiency (Totter and Day, 1942; Albanese and Buschke, 1942; Buschke, 1943; Albanese, 1945), lysine deficiency (Hock, Hall, Pund, and Sydenstricker, 1945), and methionine and protein deficiency (Sydenstricker, Hall, Hock, and Pund, 1946). In all these conditions, however, the vascularization is superficial and conjunctival oedema has been noted in association; it is possible that the deficiencies lead, through some common metabolic path, to epithelial dystrophy. This would result in an interference with deturgescence, particularly anteriorly, and a superficial vascular ingrowth would follow.

Clinical Considerations and the Role of Cortisone.—If the hypothesis that vascularization results from decreased corneal compactness and " suboxidation " were correct, new vessel formation would depend upon fluctuations in corneal thickness, since the factor of " suboxidation " is relatively constant. This in turn will depend to a great extent upon pathological changes at the limbus and in the corneal epithelium and endothelium. In some cases of corneal vascularization, only one of these factors may be involved, but in others, two, or all three, may be incriminated, the final extent and pattern of vascularization being the result of their interplay.

For instance, the clinical counterpart of the experiments in which endothelial abrasion gave rise to a corneal haze with peripheral clearing and no vascularization is seen in cases of disciform keratitis, buphthalmos, and corneal injury leading to epithelial or endothelial damage. The experiments involving more severe endothelial injury with diffuse opacification and vascularization are paralleled in keratitis profunda (uveo-keratitis) wherein severe toxic endothelial degeneration occurs.

It is doubtful whether limbal congestion alone can sufficiently interfere with the mechanism of deturgescence to give rise to vascular ingrowth. Although Wise (1943) believes that the formation of new vessels in the cornea in acne rosacea arises in response to limbal engorgement, as part of a general facial vaso-dilatation, it is probable that the process is in fact a less simple one. It is well recognized that ciliary congestion *per se*, even if of considerable duration, is not usually associated with corneal vascularization. The limbal vessels, however, may be primarily responsible for vascularization in those intense inflammatory reactions which lead to such a gross outpouring of exudate into the corneal stroma that the dehydrating mechanism is overwhelmed.

It would thus appear that the vascularization induced primarily by an inflammatory reaction at the limbus and that induced by the loss of integrity of the corneal limiting membranes, subserve two entirely different functions:

(1) In the former, the new vessel invasion represents an extension of the inflammatory process into the corneal tissue, and only in the later stages, when the aetiological factors underlying the inflammation are on the wane, will the intracorneal vessels take up a reparative role. In such cases, therefore, an inhibition of the vascular invasion is essential if the cornea is to be spared. The beneficial result following such an inhibition is strikingly shown by the results of cortisone therapy in cases of deep keratitis. A rapid reduction of the inflammatory phenomena is followed by both a decrease in corneal swelling and a clearing of the associated corneal infiltration.

(2) In the case of vascularization induced by injury of the corneal limiting membranes, however, the role played by any resulting vascularization has a beneficial and reparative effect, for it apparently assists in draining away the corneal oedema. It is well recognized that clearing of the cornea is especially rapid in those cases which show vascularization. Any success gained by cortisone therapy, therefore, is rather to be attributed to its suppression of the primary intra-ocular inflammation which allows endothelial regeneration and restoration of normal deturgescence.

The close relationship between the degree of corneal swelling and vascularization in man, has been demonstrated by Cook and Langham (1953), who measured the corneal thickness in a variety of corneal diseases, according to the technique of Maurice and Giardini (1951); their findings are illustrated elsewhere in this issue.

In conclusion, it may be of value to review the possible factors involved in the suppressive effect which cortisone is able to exert on corneal vascularization. It may achieve this result:

(*i*) by inhibiting new vessel growth.
(*ii*) by preventing a decrease in corneal compactness.

(*i*) Regarding the first possibility, it has been suggested that cortisone may suppress endothelial proliferation (Duke-Elder and Ashton, 1951); this view received some indirect support from our experiments with cortisone in the healing of corneal wounds, that there was a marked inhibition in regeneration of the corneal endothelium (Ashton and Cook, 1951). It is to be remembered, however, that the endothelial cells of the cornea are peculiar in that they secrete Descemet's membrane, a function which no other endothelium appears to possess, and it has been suggested on other grounds that they are not truly endothelial cells (Sondermann, 1930, 1949). Whatever be the cause of the inhibition in this instance,

it may not be justifiable to relate it to that of the vascular endothelium. In order to elucidate this point we have studied the effect of cortisone upon the ingrowth of new vessels into transparent rabbit-ear chambers (Ashton and Cook, 1952). The most striking features noted in the vascularizing test chambers were generalized vascular constriction, increased translucency of the vessel walls, and an exaggerated tonic response to extraneous stimuli. In the animals treated with cortisone there was a moderate delay in vascularization, to some extent more apparent than real, since the majority of the capillary buds and the vessels from which they arose were exsanguinated. Endothelial proliferation and canalization of the newly-formed buds occurred as in the control animals. We concluded that cortisone was able to inhibit vascularization in the rabbit-ear chamber, but that this influence was more probably due to its profound effect upon the circulation than to a direct inhibition of endothelial growth. The site and mode of the action of cortisone in the production of these vascular changes are as yet unknown. Reasons were given for suggesting that future enquiry might profitably be directed towards a study of the influence of cortisone upon vasomotor mechanisms. Applying these observations to the suppressive action of cortisone upon corneal vascularization, it will be seen that a direct inhibition of new vessel growth, through the mechanism described above, is probably an important factor. Indeed, the vasoconstrictive effect of cortisone on corneal vessels can be readily demonstrated clinically (Figs 9 and 10). The vasoconstrictive effect is not,

FIG. 9.—Superficial keratitis. Cornea shows marked superficial vascularization. Compare with Fig. 10.

FIG. 10.—Same cornea as shown in Fig. 9 after six subconjunctival injections of cortisone over a period of 3 weeks. Note marked vasoconstriction.

however, an immediate one; it gradually develops and increases as the period of cortisone administration extends. Langham (1952 a) holds the view that cortisone has no direct effect upon capillary proliferation, but this influence might have become apparent had his test rabbits been subjected to cortisone for a longer period.

(*ii*) Turning to the second possibility, we may quote the experimental work of Langham (1952a), who studied the effect of cortisone upon the increase in corneal thickness which follows the intracameral injection of alloxan, and demonstrated the ability of cortisone to restrain a swelling of the cornea. It is not known how cortisone achieves this effect, but, in view of the close association between corneal swelling and vascularization, there can be little doubt that this action is a potent factor, if not the most significant one, in the inhibition of corneal vascularization by cortisone. Furthermore, it is known that

cortisone is able to prevent an increase in capillary permeability (Cook and MacDonald, 1951), and this effect would play an important part in preventing the extension of exudate from the limbal vessels into the corneal stroma. Whether cortisone similarly influences the permeability of the corneal endothelium is a question which has not yet been investigated. Clearly, if it had such an action, it could affect fluid exchanges through this membrane and so alter the degree of thickness. It might further be argued that cortisone modifies corneal vascularization by damping down the severity of the inflammatory reaction within the corneal tissue, but it is not yet clear what factors, besides vasoconstriction and depression of capillary permeability, are involved in this process.

Summary

The theories which have been advanced to explain the mechanism of corneal vascularization are critically reviewed, and it is concluded that not one is wholly satisfactory. Further experiments have been carried out, in an endeavour to clarify the present position, and the findings are discussed in relation to current concepts, clinical implications, and the therapeutic action of cortisone.

No evidence has been found to support the idea that vascularization occurs in response to the release of a specific vascularizing substance in the diseased cornea, but the findings do not exclude this possibility. On the other hand, the results fully support the hypothesis of Cogan (1949a) that a reduction in the corneal compactness in the region of the limbus is a necessary stage in the process of corneal vascularization. We do not, however, agree with the view that an increase in corneal thickness is the stimulus to new vessel formation, but regard it merely as a removal of the compactness which normally obstructs their ingrowth. It is possible that a number of different mechanisms may be involved in corneal vascularization, and it is suggested as a possible hypothesis that the stimulus to new vessel growth in the cornea may be attributable to its peculiar metabolism, which results in a constant state of "*suboxidation*" in the normal corneal tissue; so that when there is a decrease in tissue compactness, vascularization will regularly occur, providing the swelling is adjacent to the limbus and is maintained for a sufficiently long period.

Since the factor of "suboxidation" is relatively constant, new vessel formation will depend upon the fluctuations in corneal thickness, which result to a great extent from the interplay of pathological changes at the limbus and in the corneal epithelium and endothelium.

The significance of vascularization induced primarily by an inflammatory reaction at the limbus is contrasted with the significance of that arising through loss of integrity of the corneal limiting membranes, and analogies are made between experimental results and clinical appearances.

The role of cortisone in the inhibition of corneal vascularization is discussed. Reasons are advanced for believing that this effect is attributable partly to its ability to promote vasomotor tone and prevent an increase in capillary permeability, and partly to its inhibitory effect on corneal swelling.

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REFERENCES

ALBANESE, A. A. (1945). *Science*, **101**, 619.
——— and BUSCHKE, W. (1942). *Ibid.*, **95**, 584.
ASHTON, N. and COOK, C. (1951). *British Journal of Ophthalmology*, **35**, 708.
——— ——— (1952). *Brit. J. exp. Path.*, **33**, 445.
——— ——— and LANGHAM, M. (1951). *British Journal of Ophthalmology*, **35**, 718.
BAKER, L. E. (1929). *J. exp. Med.*, **49**, 163.
BESSEY, O. A., and WOLBACH, S. B. (1939). *Ibid.*, **69**, 1.
BRUES, A. M., SUBBAROW, Y., JACKSON, E. B., and AUB, J. C. (1940). *Ibid.*, **71**, 423.
BUSCHKE, W. (1943). *Arch. Ophthal., Chicago*, **30**, 735.
BYERLY, T. C. (1926). *Anat. Rec.*, **32**, 249.
CAMERON, G. (1935). "Essentials of Tissue Culture Technique". Farrer and Rinehart, New York.
CAMPBELL, F. W. (1951). *Trans. ophthal. Soc. U.K.*, **71**, 287.
——— and FERGUSON, I. D. (1950). *British Journal of Ophthalmology*, **34**, 329.
——— and MICHAELSON, I. C. (1949). *Ibid.*, **33**, 248.
CARREL, A. (1922). *J. exp. Med.*, **36**, 385.
——— and BAKER, L. E. (1926). *Ibid.*, **44**, 503.
——— and EBELING, A. H. (1928). *Ibid.*, **48**, 105.
CHAMPY, C., and LOUVEL, J. (1938). *Arch. Mal. Coeur*, **31**, 705.
COGAN, D. G. (1949a). *Arch. Ophthal., Chicago*, **41**, 406.
——— (1949b). *Ophthalmologica, Basel*, **118**, 440.
COOK, C. and LANGHAM, M. E. (1953). *British Journal of Ophthalmology*, **36**.
——— and MACDONALD, R. K. (1951). *British Journal of Ophthalmology*, **35**, 730.
CROSSE, V. M., and EVANS, P. J. (1952). *Arch. Ophthal., Chicago*, **48**, 83.
DAVIDSON, J. N. (1943). *Edinb. Med. J.*, **50**, 70.
DAVSON, H. (1949). *British Journal of Ophthalmology*, **33**, 175.
DREW, A. H. (1927). *Brit. J. exp. Path.*, **8**, 176.
DUKE-ELDER, S., and ASHTON, N. (1951). *British Journal of Ophthalmology*, **35**, 695.
ENGLISH, J., BONNER, J. and HAAGEN-SMIT, A. J. (1939). *J. Amer. chem. Soc.*, **61**, 3434.
FISCHER, A. (1930). "Gewebezüchtung." 3rd ed. p. 327. Müller and Steinicke, Munich.
——— (1939). *Nature, Lond.*, **144**, 113.
FISCHER, F. P. (1927). *Arch. Augenheilk.*, **98**, 41.
——— (1933). *Ibid.*, **107**, 295.
GRUBER, R. (1894). *v. Graefes Arch. Ophthal.*, **40**, pt. 4, p. 25.
HABERLANDT, G. (1922). *Biol. Zbl.*, **42**, 145.
HAESSLER, F. H. (1927). *Trans. Amer. ophthal. Soc.*, **25**, 412.
HAMMETT, F. S. (1929). *Proc. Amer. phil. Soc.*, **68**, 151.
HEATON, T. B. (1926). *J. Path. Bact.*, **29**, 293.
HOCK, C. W., HALL, W. K., PUND, E. R. and SYDENSTRICKER, V. P. (1945). *Fed. Proc.*, **4**, 155.
INGALLS, T. H. (1948). *Pediatrics*, **1**, 315.
——— TEDESCHI, C. G., and HELPERN, M. M. (1952). *Amer. J. Ophthal.*, **35**, 311.
JOHNSON, L. V., and ECKARDT, R. E. (1940). *Arch. Ophthal., Chicago*, **23**, 899.
JONES, I. S., and MEYER, K. (1950). *Proc. Soc. exp. Biol., N.Y.*, **74**, 102.
JULIANELLE, L. A., and LAMB, H. D. (1934). *Amer. J. Ophthal.*, **17**, 916.
KJAER, S. (1928). *Arch. klin. Chir.*, **149**, 146.
KINSEY, V. E., and COGAN, D. G. (1942). *Arch. Ophthal., Chicago*, **28**, 449.
LANGHAM, M. (1952a). *Trans. ophthal. Soc. U.K.*, **72**, 253.
——— (1952b). *J. Physiol., Lond.*, **117**, 461.
MAURICE, D. M. (1951). *Ibid.*, **112**, 367.
——— and GIARDINI, A. A. (1951). *British Journal of Ophthalmology*, **35**, 169, and 791.
MENKIN, V. (1941). *Cancer Res.*, **1**, 548.
MEYER, K., and CHAFFEE, E. (1940a). *Amer. J. Ophthal.*, **23**, 1320.
——— ——— (1940b). *Proc. Soc. exp. Biol., N.Y.*, **43**, 487.
MICHAELSON, I. C. (1948). *Trans. ophthal. Soc. U.K.*, **68**, 137.
OFFRET, G., and CHAUVET, P. (1950). *Arch. Ophtal.*, **10**, 344.
PIRIE, A., SCHMIDT, G., and WATERS, J. W. (1948). *British Journal of Ophthalmology*, **32**, 321.
POTTS, A. M., and JOHNSON, L. V. (1950). *Amer. J. Ophthal.*, **33**, 405.
RONES, B. (1940). *Arch. Ophthal., Chicago*, **24**, 374.
SONDERMANN, R. (1930). *v. Graefes Arch. Ophthal.*, **125**, 407.
——— (1949). *Ibid.*, **149**, 690.
SWINDLE, P. F. (1938). *Arch. Ophthal., Chicago*, **20**, 974.
SYDENSTRICKER, V. P., HALL, W. K., HOCK, C. W., and PUND, E. R. (1946). *Science*, **103**, 194.
SZEWCZYK, T. (1952). *Amer. J. Ophthal.*, **35**, 301.
TOTTER, J. R., and DAY, P. L. (1942). *J. Nutr.*, **24**, 159.
WINDLE, W. F., BECKER, R. F., and WEIL, A. (1944). *J. Neuropath. exp. Neurol.*, **3**, 224.
WISE, G. (1943). *Amer. J. Ophthal.*, **26**, 591.
WISLOCKI, G. B., BUNTING, H., and DEMPSEY, E. W. (1947). *Amer. J. Anat.*, **81**, 1.
WOODIN, A. M. (1950). *British Journal of Ophthalmology*, **34**, 375.

Brit. J. Ophthal. (1953) 37, 210.

OBSERVATIONS ON THE GROWTH OF BLOOD VESSELS INTO THE CORNEA. APPLICATION OF A NEW EXPERIMENTAL TECHNIQUE

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FEW subjects in opthalmology are more important from the clinical point of view than opacification and vascularization of the cornea; moreover, the problems included have a wide physiological and pathological interest. The unsatisfactory state of our present knowledge of the fundamental processes involved has been pointed out in the foregoing article by Ashton and Cook (1953), who also give a review of the literature. The present paper describes experimental investigations undertaken to elucidate these matters further.

A new method of inducing a swelling and subsequent growth of vessels into the cornea has presented a unique opportunity for studying the underlying causes of these pathological phenomena. It has been shown (Langham, 1951) that a neutralized solution of the organic compound alloxan, when brought into contact with the cornea of the rabbit, can be used to initiate either a completely reversible swelling of the cornea with little or no vascularization, or a swelling followed by a dense ingrowth of vessels around the whole periphery of the cornea.

This method has been applied to the present problem in three ways:

(1) The study of the swelling and hydration of the cornea in relation to the growth of new vessels;
(2) The study of changes in metabolism and respiration following application of alloxan and preceding the growth of new vessels;
(3) The study of changes in permeability.

Methods

Adult rabbits weighing between 1·9 and 2·5 kg. were chosen for the experimental work; where required, nembutal solutions and ethyl chloride were used for anaesthesia. Fresh alloxan monohydrate was dissolved in distilled water, neutralized, and made up to an isotonic concentration, and then, if necessary, diluted with a solution of isotonic saline. The thickness of the cornea was measured by the optical device described by Maurice and Giardini (1951a). Corneal thickness was determined before beginning the experiments, rabbits being rejected if a significant difference in readings was found between the two eyes.

For the administration of the alloxan solution the animals were anaesthetized with intravenous nembutal. No anaesthetic was applied to the cornea. A hypodermic needle was introduced *via* the sclera to avoid corneal damage, and a known volume of alloxan solution was then mixed with the aqueous humour by rapid movement of the plunger of the syringe twelve times. After withdrawal of the

needle, the exterior of the cornea was well washed with warm isotonic saline. All subsequent readings of the corneal thickness and the extent of vascularization were made on the conscious animal.

Analyses.—Lactic acid was determined by the method of Barker and Summersen (1941). Tissue proteins were denatured in an 8 per cent. solution of trichloroacetic acid. For corneal analysis precautions were taken to minimize *post-mortem* glycolysis, the corneae being excised, weighed, and denatured within one minute of the initiation of excision from the unconscious animal. Estimations, which included a standard made on the day of the experiment, were carried out immediately on completion of the experiment; this was found to be necessary because of the instability of lactic acid in aqueous solution. A standard constant-volume type of Warburg manometric apparatus was used for the determination of oxygen uptake and rates of anaerobic and aerobic glycolysis. For the determination of oxygen uptake, the centre well was filled with 0·2 ml. 20 per cent. solution of potassium hydroxide, and 0·5 ml. water was placed in the side arm to prevent hydration of tissue. No suspending medium was used (deRoetth, 1950; Langham, 1952). All determinations were carried out at 37°C., using air as the gas phase. For the determination of the rate of aerobic glycolysis a Krebs bicarbonate medium containing 100 mg. per cent. glucose was used with gas mixtures of 5 per cent. CO_2 and 95 per cent. O_2.

Swelling, Opacification, and Vascularization of the Cornea

The discovery of Dunn, Sheehan, and McLetcher (1943) that alloxan administered intravenously to rabbits led to a destruction of cells in the islets of Langerhans and to a state of diabetes has led to much pathological and physiological research on its mode and mechanism of action. Two of the most striking observations concern the very rapid manner in which this organic compound induces pathological changes in the islet cells of the pancreas, and the very fast rate at which this substance is destroyed in the body. Thus, after an intravenous dose of alloxan, lesions in the beta cells of the islets are apparent within 5 min. (Banerjee, 1944; Hughes, Ware, and Young, 1944), and after this time alloxan is no longer demonstrable in the blood (Archibold, 1945; Brückmann, 1946; Karrer, Koller, and Sturzinger, 1945; Leech and Bailey, 1945). These two characteristics are important to the present study, for it follows that the corneal changes observed depend upon the immediate action of alloxan, and, further, it would seem that alloxan moves rapidly into the tissue cells.

In preliminary experiments, solutions of alloxan were brought into contact with the cornea by three methods:

(*i*) continuous drip on to the epithelial surface,
(*ii*) intra-lamellar injection,
(*iii*) direct mixing with the aqueous humour.

Swelling of the cornea resulted in all cases, but it soon became apparent that the anterior chamber was the most suitable site as it presents a simple means of bringing a given amount of alloxan into direct contact with the cornea. Unless otherwise stated, this route of administration was used in all subsequent experiments. The average volume of the anterior chamber of adult rabbits is 0·25 ml., and, by mixing this volume with 0·25 ml. of a solution of alloxan, a known amount and concentration of alloxan may readily be introduced. The rate of flow of aqueous

humour in the normal rabbit is of the order of 1 per cent. per minute, which means that the concentration of a substance in the anterior chamber will drop by at least 50 per cent. in less than one hour. This fact, together with the knowledge that alloxan injected intravenously is known to be destroyed within a few minutes, indicates that the percentage of injected alloxan diffusing into the cornea is small.

In the first series of experiments, the effect of increasing concentrations of alloxan on the living cornea was observed; all solutions were neutralized and brought to an isotonic concentration by the addition of sodium chloride. Solutions of isotonic sodium chloride or very low concentrations of alloxan gave no significant degree of corneal swelling. In the range of 0·01 to 0·05M (175 to 775γ), however, an appreciable swelling of the cornea was induced (Fig. 1). The swelling occurred over the whole area of the cornea and increased in degree during the first 3 to 4 days, but then gradually decreased with a subsequent return to the initial thickness. The range of corneal swelling varied appreciably between groups of experiments made on different days, although care was taken to use freshly prepared solutions of alloxan; no reason could be found for this variation. No generalized vascularization of the cornea was observed in these experiments, although in certain cases there was a small ingrowth of vessels appearing at the 5th to the 7th day. With the return of the main mass of the cornea to normal thickness, the blood in these newly-formed vessels slowly disappeared leaving an apparently normal cornea. A decrease in transparency was observed with the initiation of the swelling of the cornea, but full recovery was attained when the swelling had decreased to 10–15 per cent. above normal.

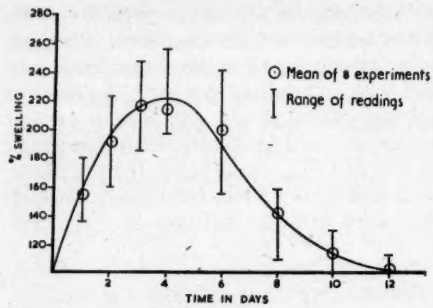


FIG. 1.—Increase in thickness of cornea after administration of 0·05M solution of alloxan into anterior chamber.

When the concentration of alloxan was raised to 0·15M, a swelling of the cornea followed by a profuse vascularization was observed. The rate of increase of thickness of the central area of the cornea after the injection of alloxan is recorded in Fig. 2. The swelling of the cornea continued until it was approximately 2½ to 3 times normal (24–48 hrs after the initiation of the experiment); with the subsequent invasion of the cornea by new vessels, no significant reduction in corneal swelling was noted, although much later, between the 6th and 13th week, the thickness very slowly returned to nearly normal.

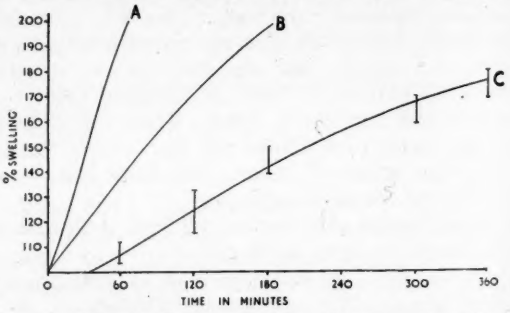


FIG. 2.—Effect of alloxan on swelling of cornea. Curve C = minimal vascularizing lesion.
Curve B (swelling of cornea after removal of epithelium) and Curve A (swelling of cornea after removal of epithelium and endothelium) are included for comparison (Maurice and Giardini, 1951b.).

The changes occurring in the blood vessels at the limbus are illustrated in Fig. 3. By microscopical examination, the small capillaries forming the limbal arcade are seen to have small, apparently dead-end, branches passing into the normal clear corneal substance. With a swelling of the cornea, these end-branches filled with flowing blood forming a cobblestone-shaped reticulum of interconnecting vessels. A marked engorgement of the episcleral and conjunctival vessels was seen on the 4th day and at this stage the small vessels showed signs of breaking up, leaving many small haemorrhages. During the next 24–48 hrs the growth of new vessels began, rapidly pushing inwards from the whole periphery towards the centre of the cornea. By the 8th day the engorgement of the perilimbal vessels had decreased, and the eye appeared much whiter; the zone of haemorrhage had also disappeared, the advancing front of new vessels being characterized by numerous small discrete spicules. Sometimes these spicules appeared isolated from the main vessels, but careful examination with the slit-lamp revealed that they were interconnected by avascular channels, which in due course filled with blood; no diffuse haemorrhage could be identified at this stage. By the 10th to 12th day, the number of small vessels near the limbus had decreased, being replaced by larger trunk-like vessels. A connection of vessels between layers of the cornea leading to a continuous flow of blood through the resulting circuits could now be distinguished. Vessel growth ceased by the 25th to 30th day, the cornea having become completely vascularized. During the following weeks, the corneal nerves appeared to increase in size and became readily apparent by retro-illumination. By the 8th week, the corneal thickness had begun to decrease, and by approximately the 16th week the cornea was only a little thicker than normal. The observation that the return of the cornea to normal thickness does not directly lead to an extrusion of blood from the vessels was made repeatedly, both in this and in the previous series of experiments.

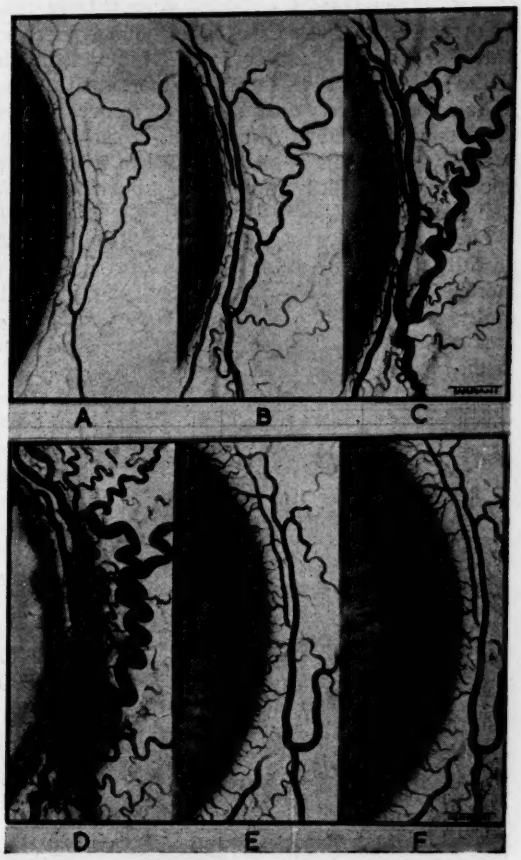
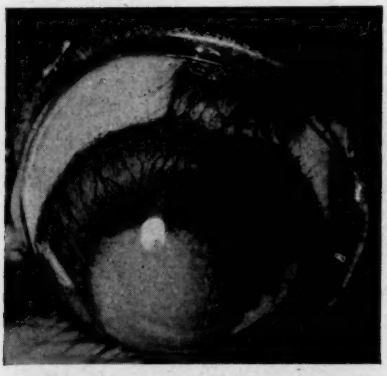


FIG. 3.—Changes in area of the corneo-scleral junction after inducement of a minimal vascularizing lesion. All drawings taken direct from the living animal.

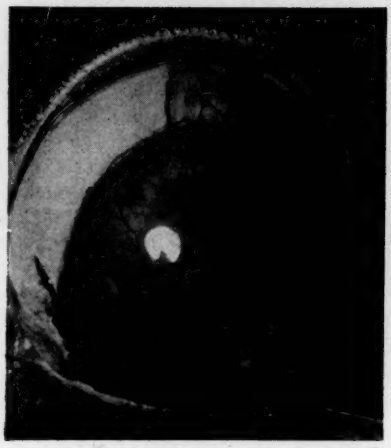
A–F normal cornea and changes 24 48, 96, 168, and 240 hrs after administration of alloxan.

These experiments describe the effect of a varying concentration of alloxan on the cornea, and indicate the changes that may precede, and occur during, a vascularization of the mammalian cornea. Of interest are the findings, first, that the cornea of the rabbit may swell rapidly to more than twice the normal thickness, and in so doing become very opaque and yet recover rapidly to its initial thickness before the extrusion of blood from vessels in the cornea; and second, that vessels will begin to grow into a swollen cornea which is just beginning to recover its normal thickness, and that cessation of this growth occurs with the thickness of the cornea well above normal.

The finding that the growth of vessels into a swollen cornea may be halted in an early phase of the recovery of the cornea to its normal condition prompted a more detailed investigation of what happens when the vessels tend to meet in the centre of the cornea. Clinically, a vascularization extending across the central area of the cornea is not infrequently observed. Similar observations were made in the case of the alloxan lesion when the rate of growth of vessels around the perimeter of the cornea varied; in contrast, a series of experiments was made in which particular care was taken to induce an even vascularization. The needle injected through scleral tissue was brought right behind the centre of the cornea and the alloxan solution mixed carefully with the aqueous humour. With the inducement of vascularization, the rabbits were examined to see if the rate of growth of vessels was uniform around the whole periphery and if this was not so they were rejected (Fig. 4*a–c*). At approximately the 25th to 30th day, the growth of vessels had



(*a*)



(*b*)

(*c*)

FIG. 4.—Inducement of an even overall invasion of the cornea by new blood vessels. (*a*) 13th, (*b*) 19th, and (*c*) 22nd day after administration of alloxan.

ceased, leaving a central area of cornea of approximately 2 mm. radius completely devoid of vessels. Structurally, this central area was, like the vascularized area, grossly altered, its thickness being approximately 2½ times normal. Thus a marked thickening of the central area of the cornea can occur without becoming vascularized.

In a final series of experiments, the effects already described (*i.e.*, a reversible swelling of the cornea returning to normal thickness within approximately 10 days, and a vascularizing lesion), were combined in the same eye by an injection of alloxan made directly into the cornea. 0.5 ml. neutralized isotonic solution of alloxan of varying concentration was injected into the corneal stroma on the temporal side, and a bleb produced extending to the limbal area. Swelling of the cornea occurred with all dilutions of alloxan, but below a concentration of 0·15M no vascularization ensued. With a solution of 0·31M, vascularization from points around the whole periphery occurred, even though by the 10th to 15th day the nasal half of the cornea was decreasing in thickness. By the 20th day, the nasal halt of the cornea had quite returned to normal thickness, yet the growth of blood vessels towards the centre of the lesion continued on the temporal side. In the following weeks the flow of blood through the vessels in the nasal half of the cornea gradually diminished, and, as suggested by their increasingly anaemic appearance, the vessels became smaller until by approximately the 10th to 13th week no more blood could be seen in the vessels. The pattern of vessel growth produced by the lesion is illustrated in Fig. 5*a* and *b*. It was observed that the growth of vessels was directed towards the site of the lesion, branches from the

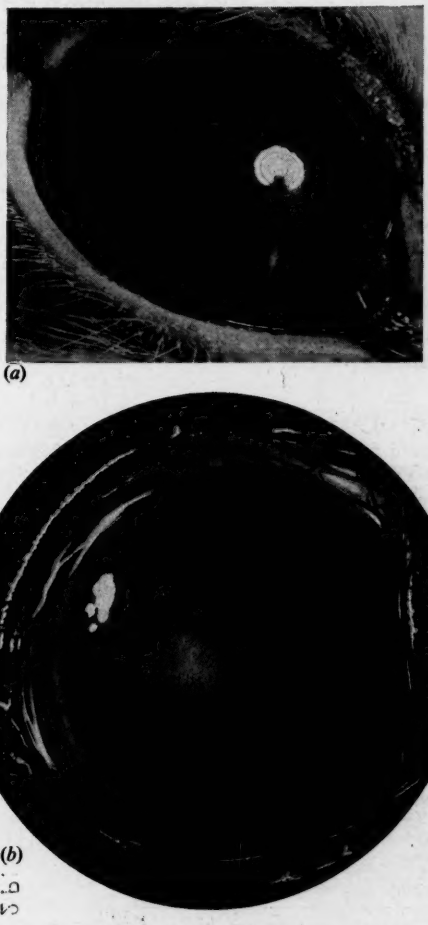


FIG. 5.—(*a*) Photograph and (*b*) painting of corneal vascularization illustrating directional properties of growth. Note that nasal side of cornea has returned to normal thickness and transparency, yet growth of blood vessels through the area continues.

main vascular trunks being more numerous on the side nearest the lesion. The cornea around the site of the lesion and extending to the limbus remained markedly swollen (greater than 2½ times normal thickness), and yet the vascularization in this area remained sparse.

Effect of Alloxan on Corneal Glycolysis and Respiration

In normal rabbits it has been demonstrated that the concentration of lactic acid in the two corneae of the same animal is equal (Langham, 1952). The effect of alloxan on the concentration of lactic acid in the cornea of the living rabbit is recorded in Table I. The minimal dose of alloxan required to induce a vascularizing lesion of the cornea was injected into the aqueous humour of one eye of each of a group of eighteen rabbits. Four of these experimental animals were chosen at random and kept as controls; the corneae of the rest were excised after various intervals of time, weighed, and analysed for lactic acid. It will be seen from a comparison of the weights of the normal and experimental corneae (columns 1 and 2 of Table I) that corneal swelling increased from a time less than one hour after administration of the compound. In contrast with the steady increase in corneal swelling, the concentration of lactic acid in the experimental cornea approached normal after 24–48 hrs. The initial drop in concentration of lactic acid could be due either to an inhibition of corneal glycolysis or to an increased diffusion of lactic acid from the cornea. That it is caused by an inhibition of corneal glycolysis is suggested by the return of the concentration of lactic acid to normal by the 24th to 28th hour, at which time corneal swelling has become very marked.

TABLE I

EFFECT OF ALLOXAN ON CONCENTRATION OF LACTIC ACID IN LIVING CORNEAE

2·5 mg. alloxan dissolved in a neutralized isotonic solution was injected into the anterior chamber of the experimental eye, and after a given time both corneae were removed for analysis.

Corneae (mg. wet weight)		Time (hrs)	Lactic Acid Concentration (mg. per cent. wet weight)		Ratio Experimental/Control
Control	Experimental		Control	Experimental	
86	94·5	1	80·3	48·7	0·605
91·4	102·8	1	83·1	32·2	0·385
79·7	106·6	2	73·3	52·7	0·716
112·7	121·1	2	98·7	56·9	0·575
93·3	179·6	24	70·3	52·6	0·745
71·6	144·3	24	74·4	77·0	1·05
66·2	132·7	24	78·6	74·8	0·955
101·3	171·5	24	69·5	70·1	1·01
77·3	152·6	24	71·2	54·7	0·77
93·1	139·4	48	42·4	70·0	1·65
79·4	200·1	180	83·5	48·2	0·578
83·6	199·1	180	102·7	70·8	0·690
80·0	217·2	196	84·6	63·7	0·754
78	289·5	196	86·5	50·5	0·585

In order to obtain more definite information on this point, it was decided to test the effect of alloxan on the accumulation of lactic acid in the excised cornea. Normal corneae were removed from unconscious rabbits, and one was added to 2·5 ml. of a Kreb's Ringer phosphate solution containing glucose to a concentration of 100 mg. per cent. while the second was placed in 2·5 ml. of a similar solution to which had been added 0·1 ml. of a neutralized solution of freshly prepared alloxan to give a concentration of 100 mg. per cent.

alloxan in the total solution. The flasks containing the corneae were then shaken for a period of one hour in a water bath at 37°C. using air as the gas phase, after which time the solution plus corneae were analysed for lactic acid. In this experiment alloxan was found to cause a marked reduction in the concentration of lactic acid in the cornea, the ratio of concentration of lactic acid in the experimental corneae to that in the control corneae being $0 \cdot 605 \pm 0 \cdot 02$ after 60 min. incubation (4). This ratio includes the amount of lactic acid originally in the corneae, and in consequence inhibition of the production of lactic acid will be greater than the ratio suggests. Thus, the rate of corneal glycolysis in the living rabbit is reduced after the injection of alloxan into the anterior chamber.

Assuming that there is an initial reduction in corneal glycolysis, further scrutiny of Table I clearly indicates that its effect is temporary, for the concentration of lactic acid in the experimental cornea rises to a similar value to that found in the control cornea in spite of the marked corneal swelling. To test whether the rate of glycolysis does in fact return to normal, a manometric determination was made of the maximal rate of aerobic glycolysis on the excised tissue between the 24th and 48th hour after administration of alloxan. The values based on the dry weight of the cornea, indicate that the rates of glycolysis in the two corneae do not differ significantly (Table II). It follows that alloxan does cause an initial inhibition of glycolysis, which is reversed with the disappearance of alloxan from the cornea. Table I shows that, with inducement of new vessel growth into the cornea, the concentration of lactic acid dropped again, an effect which may well be due to a more easy and rapid loss into the blood circulation.

TABLE II

AEROBIC GLYCOLYSIS OF CORNEAE EXCISED AFTER ADMINISTRATION OF ALLOXAN

Corneae (mg. wet weight)		Time (hrs)	µl CO_2 evolved per mg. Dry Tissue per hr		Ratio Experimental/Control
Control	Experimental		Control	Experimental	
74·3	202·2	24	1·2	0·9	0·75
92·8	173	24	1·6	1·5	0·935
87·0	146·5	24	1·0	1·1	1·1
80·0	197·5	48	1·47	1·22	0·83
78·0	131	48	1·12	1·28	1·14
85·0	231	48	1·31	1·1	0·84

The effect of alloxan on the oxygen uptake of the living cornea was determined by removal of the corneae at various times after the administration of alloxan; the rates of oxygen uptake are recorded in Table III.

TABLE III

OXYGEN UPTAKE OF CORNEAE EXCISED AFTER ADMINISTRATION OF ALLOXAN

Corneae (mg. wet weight)		Time (hrs)	µl Oxygen Uptake per mg. Dry Tissue per hr		Ratio Experimental/Control
Control	Experimental		Control	Experimental	
73·0	183·9	24	0·84	0·65	0·77
73·5	177·0	24	0·925	0·71	0·765
83·3	133·6	24	0·60	0·44	0·73
91·5	157·4	120	0·70	0·20	0·286
61·1	130·8	120	0·91	0·50	0·55
80·0	261·7	120	0·77	0·31	0·40

It is clear that in these experimental conditions alloxan does lead to a marked and prolonged inhibition of the oxygen uptake of the cornea.

Permeability of Corneal Tissue

Gross changes in the permeability of the corneal tissue and of the capillary endothelium lining the limbal vessels of the cornea were studied, using the water-soluble vital dye Pontamine sky blue (5×); 5ml. of a 5 per cent. aqueous solution of this dye may be injected intravenously into the conscious rabbit without immediate injurious reaction; after 4 or 5 weeks, however, the animal usually dies. The dye was injected into normal rabbits and its diffusibility into the aqueous humour and cornea was studied by slit-lamp observation and aqueous humour puncture. No dye penetrated either across the blood-aqueous barrier, or into the cornea from the limbal arcades in the normal rabbit. In contrast, it was observed that the dye rapidly diffused across the walls of the conjunctival vessels and accumulated in the lymphatics of this tissue. To test the permeability of the normal corneal endothelial barrier to this dye, an isotonic solution was injected directly into the aqueous humour. After 12 hours the anterior chamber had become completely free of the dye, the lens was tinted, but the cornea, like the aqueous, was completely clear and uncoloured. Direct application to the surface of the cornea demonstrated that the normal epithelium is also impermeable to the dye. After administration of alloxan into the aqueous humour and the inducement of corneal swelling, an increase in permeability of the walls of the capillary limbal vessels occurred, as was indicated by a diffusion of the dye into the mass of the cornea.

Experiments to determine the increase in permeability of the endothelial and epithelial barrier were more difficult to assess, but it would seem that little if any dye passed across these barriers even in the swollen cornea. In confirmation of this conclusion, it was observed that fluoreseein solution applied directly on the swollen cornea caused no staining as exhibited by a damaged epithelium. In these circumstances, it might be expected that the main transfer of fluid would occur from the limbal vessels. That this is so was indicated by biomicroscopical observation of an optical section of the cornea after the administration of alloxan. The angle between the slit-lamp beam and the microscope being set at 60°, and a magnification of 40 being used, the structural components of the cornea are readily distinguished. One hour after administration of alloxan no obvious changes could be seen in the epithelium or endothelium, but a slight increase in reflected light could be seen, in the area of the stroma lying just below the epithelium and by the second hour this was more enhanced as was indicated by numerous light and dark thin lines. With this increase in corneal swelling the corpuscles became ill-defined, especially in the area immediately below the epithelium. By the end of 24 hrs, corneal corpuscles could no longer be distinguished except near the endothelial surface, while in the area adjacent to the epithelium marked cleavage planes had become apparent. Even at this stage, however, no change could be observed in the epithelium and endothelium. It follows that, in this experimental condition, fluid transfer is mainly from the limbal region.

By contrast, in a similar experiment in which the endothelium in the central area of the cornea was damaged, the structural changes observed by the slit-lamp first began in the area adjacent to the endothelium, indicating that the fluid transfer n this case was predominantly from the aqueous.

Discussion

The object of this experimental work has been to study some of the main metabolic and physical changes which may occur in a cornea whose normal function is disturbed, and to correlate these events with the phenomenon of the growth of new vessels into the cornea.

A considerable amount of research has already been devoted to the study of the water balance of the mammalian cornea (Cogan and Kinsey, 1942a, b; Kinsey and Cogan, 1942a, b; Pirie, Schmidt, and Waters, 1948; Davson, 1949; Maurice, 1951; Langham, 1952, 1953). It is generally agreed that the water content, or degree of hydration of this tissue, is maintained at a constant value by the special physical characteristics of the limiting membranes, comprising the stromal-epithelial and the stromal-endothelial barriers, the functional integrity of which depends on the active metabolism of the epithelial and endothial cells. Should the dehydrating mechanism of the cornea be unable to extract fluid fast enough to maintain its normal state, an increased hydration and swelling will result. Swelling takes place mainly in the stroma, a region which comprises by weight and thickness 90 per cent. of the whole cornea (Langham, 1952). The stroma is formed of an extracellular space of approximately 12–15 per cent. and a cellular volume occupying the remaining 85–88 per cent. In consequence, stromal swelling can be due either to an increase in hydration of both the extracellular and the intracellular phases, or to the increase of one at the expense of the other. No attempt was made in the present study to determine the relative changes occurring in these two phases during the swelling of the cornea, but it would appear that the increase in hydration occurred initially and to a greater degree in the extracellular space. Thus, slit-lamp observation of an optical section of the cornea showed a separation of groups of stromal fibres, which was especially marked in the initial stages in the region below the epithelium.

The transparency of the cornea is intimately connected with its water content, which in the cornea of the living rabbit comprises 77 per cent. of its total weight. This value is the same in the cornea of both eyes and varies little in different rabbits (Duane 1949). Although the exact nature of the mechanism whereby hydration is maintained at this specific value is not yet known, it is of interest to observe its efficiency as exemplified in the present work. A cornea swollen to considerably above twice its normal dimensions and densely opaque recovered in a few days to assume a normal thickness and clarity. Certainly the factors governing the conditions essential for the maintenance of transparency are sufficiently effective to determine rapid recovery from a lesion of this nature. This efficiency in dealing with an abnormal content of fluid is even more striking when vascularization has occurred; in these circumstances the dehydrating mechanism has to cope with abnormally great fluid-traffic, but yet the cornea may resume its normal thickness while the circulation in these vessels is still maintained.

The normal mammalian cornea, bathed posteriorly by the aqueous

humour and anteriorly by tear fluid, is devoid of vessels except in a narrow zone at its periphery. If the peripheral vessels grow into the cornea they must penetrate the extracellular phase, the permeability of which is determined mainly, if not wholly, by the state of the inter-fibrillar ground substance. This is a highly polymerized carbohydrate protein complex, which forms a viscous barrier to the diffusion of particles of relatively small molecular dimensions; thus it has been observed in the present study that the dye Pontamine sky blue does not ordinarily pass into the main mass of the stroma from the pericorneal capillaries. It would seem obvious that an invasion of the cornea by new vessels must be preceded by a decrease in the compactness of this extracellular volume. Thus it is significant that following the application of alloxan, an increased hydration of the cornea in the region of the limbal vessels is an essential pre-requisite to the ingrowth of vessels. This does not, however, preclude the possibility that in other circumstances vessels might grow into a cornea without this previous swelling; it is conceivable that a decrease in compactness might arise just as effectively from a loss of ground substance, even if the mechanism of corneal dehydration were functioning at a normal or even a super-normal level.

The difficulties of ascertaining the nature of the stimulus determining the growth of new vessels into the cornea may be gathered from the present work. After the administration of alloxan, changes in metabolism and structure precede vascularization. Specifically, the stimulus has been ascribed to asphyxia (Bessey and Wollbach, 1939; Johnson and Eckardt, 1940), to inflammation (Julianelle and Lamb, 1934), to toxins (Haessler, 1927), or to a fall in tissue tension (Cogan, 1948, 1949).

In this respect the recent work of Cogan deserves special consideration. He made small experimental lesions in the cornea of the rabbit and observed that a swelling in the region of the limbal vessels always preceded vascularization. On this and similar evidence he suggested that the vessels at the limbus are normally prevented from entering the cornea by the compactness of the tissue; should, however, this be sufficiently decreased, ingrowth of vessels would occur. He thus puts forward the view that the lowering of extravascular pressure in the cornea results in an engorgement of limbal vessels; saccular aneurysms form, which ultimately burst, and this is followed by the outgrowth of new capillaries. When the stroma becomes isotonic to the blood, and, in consequence, hypotonic to the tears and the aqueous humour, the cornea automatically returns to its normal state; in these circumstances water is withdrawn from the cornea which slowly becomes deturgesced, and, finally, blood is extruded from the corneal vessels.

In conclusion, it is of interest to consider a final point which indicates the complexity of this problem. It will be remembered that, in the reversible swelling of the cornea following the application of alloxan, after the vessels growing inwards from the periphery had reached a distance of 1 to 2 mm., they ceased to grow even although the cornea was still more than twice its

normal thickness and weight. Moreover, vascularization ceased in some instances when the pathological changes had passed their acute stage but the cornea was still swollen to twice its normal thickness. If this persistence of swelling is still confined to the extracellular phase at this stage, it would seem to follow that gross swelling of the cornea *per se* is not the sole stimulus for vascular growth. These are issues which require further study before any final conclusion can be made as to the nature of the stimulus to new vessel growth.

Summary

(1) A method is described for the inducement of a completely reversible swelling of the cornea with little or no vascularization, or of a swelling followed by a dense ingrowth of vessels around the whole periphery of the cornea. With the application of this technique, an investigation has been made of the phenomenon of the growth of new vessels into the cornea of the rabbit.

(2) The swelling and hydration of the cornea in relation to the growth of new vessels has been noted. Changes taking place in the thickness of the cornea before and during vascularization have been measured.

(3) It is shown that an increased hydration of the corneal area adjacent to the perilimbal capillaries is an essential pre-requisite to the invasion by blood vessels following the application of alloxan.

(4) Invasion of the cornea by blood vessels ceases when the swollen cornea enters a phase of recovery, even though the cornea is still greatly swollen.

(5) Extrusion of blood from vessels in the cornea does not precede the return of the cornea to a normal thickness and weight.

(6) When new vessels are growing evenly towards the centre of the cornea from the whole periphery, growth will cease leaving a small central area devoid of vessels, though grossly swollen.

(7) Changes in glycolysis and respiration which occur after the application of alloxan and before the growth of the new vessels have been measured. It has been shown that alloxan causes a marked inhibition of corneal glycolysis, but recovery ensues with disappearance of alloxan. The inhibition of respiration has been shown to be reversible.

(8) Changes in permeability preceding the growth of vessels into the cornea have been observed, using the vital dye, Pontamine sky blue. A considerable increase was noted in the permeability of the walls of the capillary vessels at the limbus of the cornea.

(9) The results are discussed in the light of present knowledge of the normal physiology of the mammalian cornea.

I should like to express my deep gratitude to the director of research, Sir Stewart Duke-Elder, for his interest and unfailing advice and help during the course of this study, and to the Medical Research Council for defraying the cost of this research. I am also indebted to Miss S. Scotchbrook for her technical assistance.

REFERENCES

ARCHIBOLD, R. M. (1945). *J. biol. Chem.*, **158**, 347.
ASHTON, N., and COOK, C. (1953). *British Journal of Ophthalmology*, **37**, 193.
——— ——— and LANGHAM, M. (1951). *Ibid.*, **35**, 718.
BANERJEE, S. (1944). *Lancet*, **2**, 658.
BARKER, S. B., and SUMMERSON, W. H. (1941). *J. biol. Chem.*, **138**, 535.
BESSEY, O. A., and WOLBACH, S. B. (1939). *J. exp. Med.*, **69**, 1.
BRÜCKMANN, G. (1946). *J. biol. Chem.*, **165**, 103.
COGAN, D. G. (1948). *Trans. Amer. ophthal. Soc.*, **46**, 457.
——— (1949). *Arch. Ophthal., Chicago*, **41**, 406.
——— and KINSEY, V. E. (1942a). *Ibid.*, **27**, 466.
——— ——— (1942b). *Ibid.*, **28**, 661.
DAVSON, H. (1949). *British Journal of Ophthalmology*, **33**, 175.
DEROETTH, A., Jr. (1950). *Arch. Ophthal., Chicago*, **44**, 666.
DUANE, T. D. (1949). *Ibid.*, **41**, 736.
DUNN, J. S., SHEEHAN, H. L., and MCLETCHIE, N. G. B. (1943). *Lancet*, **1**, 484.
HAESSLER, F. H. (1927). *Trans. Amer. ophthal. Soc.*, **25**, 412.
HUGHES, H., WARE, L. L., and YOUNG, F. G. (1944). *Lancet*, **1**, 148.
JOHNSON, L. V., and ECKARDT, R. E. (1940). *Arch. Ophthal., Chicago*, **23**, 899.
JULIANELLE, L. A., and LAMB, H. D. (1934). *Amer. J. Ophthal.*, **17**, 916.
KARRER, P., KOLLER, F., and STURZINGER, H. (1945). *Helv. chim. Acta*, **28**, 1529.
KINSEY, V. E., and COGAN, D. G. (1942a). *Arch. Ophthal., Chicago*, **28**, 272.
——— ——— (1942b). *Ibid*, **28**, 449.
LANGHAM, M. (1951). *Quoted in* Ashton, Cook, and Langham (1951).
——— (1952). *J. Physiol., Lond.*, **117**, 461.
——— (1953). *Ibid.* In the press.
LEECH, R. S., and BAILEY, C. C. (1945). *J. biol. Chem.* **157**, 525.
MAURICE, D. M. (1951). *J. Physiol., Lond.*, **112**, 367.
——— and GIARDINI, A. A. (1951a). *British Journal of Ophthalmology*, **35**, 169.
——— ——— (1951b). *Ibid.*, **35**, 791.
PIRIE, A., SCHMIDT, G., and WATERS, J. W. (1948). *Ibid.*, **32**, 321.

Brit. J. Ophthal. (1953) **37**, 223.

TECHNIQUE OF LAMELLAR SCLERAL RESECTION*

BY

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THE purpose of this paper is to describe certain points in the technique of lamellar scleral resection which I think help in its efficient performance and shorten the time spent upon it. Particular mention is made of the scleral marker, the suture-retention coil, and the suture-tying forceps, new instruments which I have designed to assist in the orderly conduct of the operation. The time taken for a half-circumference resection of a scleral strip 28 mm. long is 45–55 minutes. The resection of a strip 45 mm. long which necessitated the division of three extra-ocular muscles for adequate exposure took 73 minutes.

TECHNICAL POINTS IN THE OPERATION

HAEMOSTASIS.—Oozing of blood is much reduced by injecting 0.5 ml. saline with adrenaline chloride 1:1000 one minim into Tenon's capsule in each quadrant for resection, that is 1 ml. with 2 minims adrenaline when half the scleral circumference is marked for lamellar resection.

RETRACTION OF CONJUNCTIVAL INCISION, TENON'S CAPSULE, AND A DIVIDED RECTUS MUSCLE.—It is well to keep the posterior edge of the incision in the conjunctiva and Tenon's capsule retracted by three interrupted sutures of No. 1 black silk placed at equidistant intervals and fixed to the head drape with clips.

The four needles of the two double-armed sutures which traverse the belly of an extra-ocular muscle 2 mm. behind its site of division are passed through the head drape in their correct sequence, and then these and the conjunctival retraction sutures are covered by a small green linen towel fixed in position by clips. The operative field is thus cleared of those sutures which are not required until the close of the operation and whose presence might confuse its progress by becoming intermingled with the scleral sutures. In the case of an extensive lamellar resection of about two-thirds of the circumference of the eye, there are as many as thirty sutures in the field of operation at the same time.

MUSCLE TRACTION SUTURES.—I have found that distinctive coloured traction sutures of No. 1 silk in the tendons of the three rectus muscles exposed for a resection of half the circumference of the sclera are helpful in turning and keeping the eye in different positions during resection and suturing of the sclera. A black suture is inserted in the stump of the divided rectus muscle, a white suture into one of its neighbours and a red suture into the other. The ends of these sutures are held in a curved pressure forceps which may either rest on or be clamped to

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the head drape when the eye is turned in the desired position. By means of these sutures the position of the eye may be changed and will remain so without any need for the surgeon or his assistant to apply forceps.

RESECTION OF THE SCLERA.—The scleral marker (Fig. 1) is easily applied to the exposed sclera with its anterior edge 10 mm. posterior to the limbus, and with a mapping pen dipped in gentian violet the outline of the marker is made on the sclera (Fig. 2). Markers of various lengths and breadths may be used. This method is much quicker than repeated applications and measurements made with compass dividers.

Invaluable instruments for the resection of the sclera are:

(1) Two scleral hooks (Stallard).

(2) No. 15 Gillette blade in a No. 3 Bard Parker handle (Fig. 2).

The pointed end of the scleral hook is 1 mm. long and makes an angle of 45° with the shaft, and so it is not a curved hook. I had such hooks made for me during the war by R.E.M.E. for the retraction of scleral incisions in the removal of intra-ocular foreign bodies by the posterior route. They are much superior to the curved hooks. A hook enters the sclera about 0.5 mm. anterior to the gentian violet marking of the posterior line at one end of the ellipse for scleral resection (Fig. 2); it steadies the eye and retracts the anterior lip of the scleral incision, and as this progresses another application of the hook is made. If it is difficult to judge the depth of the incision the assistant uses the second hook for retraction of the posterior edge of the scleral incision, but usually retraction of the anterior lip is sufficient.

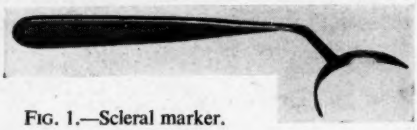

FIG. 1.—Scleral marker.

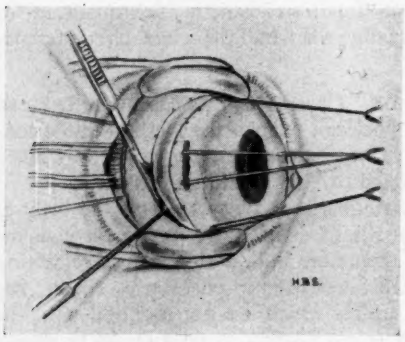

FIG. 2.—Exposure, marking, and incision of site for scleral resection.

The Gillette blade is exquisitely sharp and well finished and its belly is drawn over the sclera to a depth where a faint blue-grey colour of the choroid appears, that is to a plane where a single layer of scleral fibres remains on the choroid. It is important to keep this depth accurately throughout the incision for upon this depends the ease with which the strip of sclera is separated in the right plane.

A like procedure is carried out in the anterior line of the ellipse for incision. The two incision lines join at each tapered end of the part marked for resection. One tapered end is then grasped with Jayle's forceps and lifted whilst the belly of the Gillette knife cuts the deeper scleral fibres tangentially in the plane which leaves on the choroid a single layer of scleral fibres, and with slight tension on the raised

tongue of sclera held in the forceps the sclera separates this desired plane aided by gentle sweeps of the knife. It is important not to drag the scleral tongue so that distortion of the normal scleral curve occurs, for this might cause the knife to leave its correct tangential plane and either cut the deepest scleral layers and so expose the choroid, or to pass too superficially and so leave a thicker amount of sclera than is desired. After dissecting in this way for 1 cm. the scleral tongue is turned back on itself, and in the line of resection three or four interrupted scleral sutures are inserted; this procedure is repeated with each cm. of resection (Fig. 3).

RETENTION OF SCLERAL SUTURES.—In some cases, particularly when resections of 45 mm. are made, 22 interrupted scleral sutures, placed at 2 mm. intervals, are used to close the incision. To avoid the confusion which might result from these white sutures becoming crossed, entangled, and difficult to identify on account of blood staining, it is essential to secure at least one end of each suture. It is preferable to anchor the longer posterior end, and to leave that part of the suture which emerges from the anterior lip of the incision lying on the sclera. The latter is cut so that it is not more than 1 cm. long and so does not cross the limbus to touch the cornea.

The suture-retention coil (Fig. 4), which is 6 cm. long, is fastened to the head drape by three spring clips. Its coils act like a spring and they retain the sutures in their proper order when these are placed between them at appropriate intervals (Figs 3 and 5). The sutures thus held do not become stained with blood and tissue

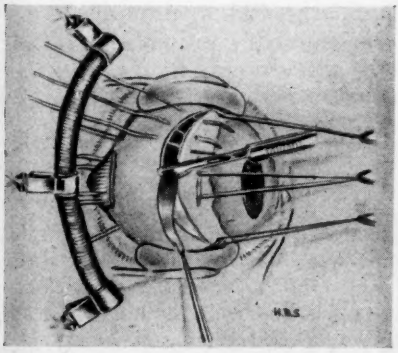

FIG. 3.—Lamellar dissection of sclera.

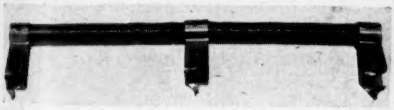

FIG. 4.—Suture-retention coil.

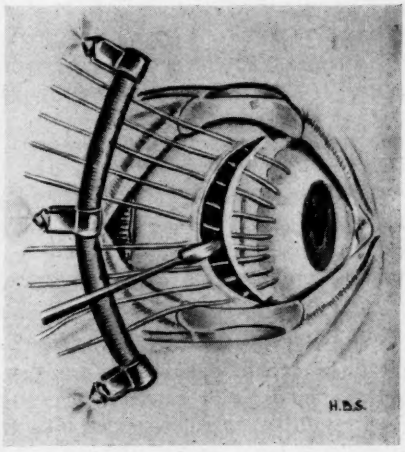

FIG. 5.—Sutures held in suture-retention coil, and application of caustic potash.

fluids. This retention device is better than the use of multiple bull-dog clips which may cross each other and cause tangling of the sutures.

TYING OF SCLERAL SUTURES.—After the resection is completed, the remaining layer of scleral fibres is lightly painted with caustic potash 3 per cent. (Fig. 5), and

the sclera and choroid are punctured by a penetrating diathermy needle over the site of the inter-retinal fluid. This may be done in the line of the resection. The inter-retinal fluid is gently sucked through the penetration and after this the intra-ocular pressure falls in most cases so that coaptation of the scleral incision is possible. The longer end of each suture is picked up from the retention coil with a pair of epilation forceps, for the sutures have been drawn through sterile liquid paraffin to facilitate their passage through the sclera and are apt to slip when seized by gloved fingers. The longer end of the suture is then wound round the curved end of a pair of suture-tying forceps, the tips of which are then opened to grasp securely the shorter end of the suture between the blocks (Fig. 6). Fig. 7 shows a pair of these suture-tying forceps made on the pattern of fine curved mosquito forceps with the inner surface of each end fitted with smooth surfaced blocks 7 mm. in length exactly opposing each other. A fine silk suture may be held securely between these closed blocks.

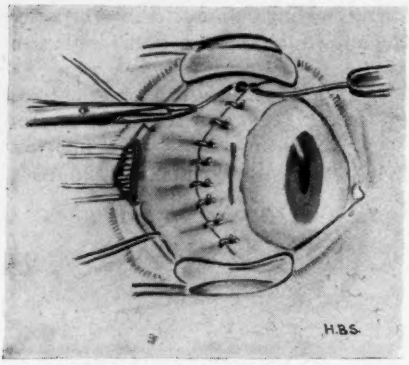

FIG. 6.—Scleral suture being tied by suture-tying forceps and epilation forceps.

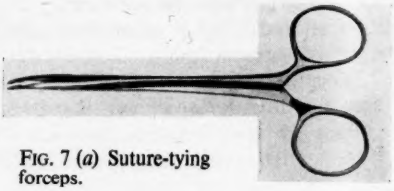

FIG. 7 (*a*) Suture-tying forceps.

FIG. 7 (*b*) Enlarged view of tips of forceps.

Interrupted sutures close the scleral incision neatly and smoothly, but mattress sutures, which are recommended by some surgeons, cause puckering of the sclera and an uneven suture line.

COMPLICATIONS

Closure of the Scleral Incision.—There is very rarely any difficulty in bringing the edges of the scleral incision together, and when this occurs paracentesis of the anterior chamber may allow a further fall in the intra-ocular pressure sufficient to tie the sutures with the scleral edges co-apted. The unpleasant sequel of vitreous forced into the anterior chamber after repeated paracentesis followed by forceful attempts to bring together the edges of the scleral incision has been reported by Shapland in full-thickness scleral resection, but I have never known this to happen in lamellar scleral resection.

Herniation of the Choroid.—If the knife blade is not kept accurately in the plane of the lamellar dissection, the deepest layer of the sclera may be sliced and the choroid exposed.. If the area of exposure is 3 mm. or more, and the manipulations have raised temporarily the intra-ocular pressure, the choroid may bulge progressively into the wound. Immediate action should be taken by puncturing the

sclera with penetrating diathermy over the site of the deepest part of the inter-retinal fluid and evacuating sufficient of this to allow the choroid to recede. It is important not to puncture the bulging choroid, for immediately behind this may lie vitreous gel and not inter-retinal fluid.

It is possible, but difficult, to continue the lamellar dissection after evacuation of some inter-retinal fluid.

Results

During 1951 and 1952 I performed this operation on 28 patients, and in two of them in both eyes, making a total of thirty eyes. The patients chosen were those with extensive retinal detachment, some of long duration, for whom the prognosis was bad on account of high myopia, aphakia, stellate folds, Eales's disease, severe trauma, and (in six cases) when one or more previous diathermy operations had failed. The majority were forlorn hopes. None of the failures were made worse by the operation and in no instance was excision of the operated eye indicated. It is probably too early to be sure that some of the successes will so remain.

Success.—In thirteen patients (fourteen eyes—one bilateral) the retina has been completely replaced. Of these cases whose operations were successful, four (5 eyes) were aphakic, five had myopia, two were emmetropic, one had severe traumatic retinal detachment of 5 years' duration, and one suffered from Eales' disease with retinal detachment associated with bands of fibrous tissue organized from blood in the vitreous. In eleven of the fourteen eyes half the circumference of the eye was resected, in one three-quarters, and in two the entire circumference. The visual result of these successes is between 6/6 and 6/24 in nine cases, and 6/60 in two cases; in the remaining three, macular changes made the vision less than 6/60.

Moderate Improvement.—Seven eyes were improved; that is the area of retinal detachment is much reduced after operation and has remained so to date but is not entirely replaced. In one instance the retina was completely replaced and remained so for 3 months before a small area became detached. In this group aphakia was present in three cases, myopia in three, and emmetropia in one. Vision improved to 6/18 in one case and to 6/36 in another.

Failure.—In eight patients (nine eyes—in one patient one eye was successful and the other a failure) the operation was a failure, the retinal detachment being as extensive as before. Of these, two have aphakia, five myopia, and two trauma. One of these last had a penetrating wound with an intra-ocular foreign body lodged on the nasal edge of the optic disc whence it was removed by the posterior route technique; he subsequently developed retinitis proliferans and total retinal detachment. In one of these failures the retina was completely replaced for six months before detachment recurred.

I think it may be stated with certainty that this operation, accurately performed, eliminates the dangers of choroidal haemorrhage and vitreous

loss, two serious hazards which may complicate and spoil the full-thickness scleral resection particularly when there are adhesions between the choroid, sclera, and retina after a previous diathermy operation. In my limited experience the results are better in this series of lamellar scleral resection than in comparable cases where the full-thickness scleral resection has been done.

Summary

Certain points in the technique of lamellar scleral resection are described. The use of new instruments, the scleral marker, suture-retention coil, and suture-tying forceps, reduce the time taken over this operation and increase its efficiency.

I thank Messrs. Stone and Jaspar of Down Bros. and Mayer and Phelps for arranging to make these instruments to my design and for their helpful advice in modifying the handle of the scleral marker.

REFERENCE

SHAPLAND, C. DEE (1951). *Trans. ophthal. Soc. U.K.*, **71**, 29.

Brit. J. Ophthal. (1953) 37, 229.

PROTEIN STUDIES ON THE HUMAN VITREOUS BODY*

BY

B. CAGIANUT AND C. WUNDERLY

From the Ophthalmological Clinic (Director: Prof. Marc Amsler) and the Medical Clinic (Director: Prof. W. Löffler) of the University of Zürich

INVESTIGATIONS of the vitreous body are beset with numerous technical difficulties. Its large water content and the instability of its structure make it difficult to obtain unchanged vitreous body in large amounts. Through the slit lamp and in fixed histological preparations (Lauber, 1936; Baurmann, 1926; Comberg, 1924; Duke-Elder, 1930), the human vitreous body appears as a structured mass.

In order to circumvent the criticism that such structures are caused artificially, new methods of investigation had to be evolved. Through the ordinary microscope fresh vitreous body demonstrates no structural elements. On the other hand, investigations with the ultramicroscope (Friedenwald and Stiehler, 1935), darkfield microscope, and phase-contrast microscope (Redslob, 1932; Matoltsy and others, 1951; Grignolo, 1952) have not led to any single conclusion, but have given rise to the present view of the vitreous body as a gel, permeated by a net of submicroscopic fibrils.

As Mörner (1893) has shown, this structural part can be split off by filtration. The chemical and physical analysis of the filter remains (Pirie and others, 1948) resulted in a collagen-type protein and a polysaccharide acid which could be identified as hyaluronic acid (Meyer and Palmer, 1936). The further structural analysis of these elements by means of the electron microscope and *x*-ray diagrams (Pirie and others, 1948; Schwarz and Schuchardt, 1950; Schwarz, 1951; Matoltsy and others, 1951) led to the discovery of three main types of fibrils:

(*i*) long unbranched threads with an indication of fine axon periods;
(*ii*) delicate, unbranched, entangled threads with a distinct axon period of 500–850Å;
(*iii*) broad, striated fibrils with an axon period of 500–800Å, which demonstrated the typical relationship of collagen.

This structural residual protein consists of various components which can be separated and demonstrated by electrophoresis. To Hesselvik (1939), we owe the thorough electrophoretic analysis of the proteins of the vitreous body. Since the introduction and development of paper-electrophoresis, it has become possible to investigate the protein composition of single

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vitreous bodies in spite of the slight amount of protein contained. The protein components of the vitreous body, like the serum proteins, have the property of binding water and thus contributing to the colloidosmotic pressure. The strength of this binding can be measured by the rapidity with which heavy water penetrates the vitreous body of the living eye (Cagianut and Verrey, 1949). With a view to determining the influence of changes of pressure, we have now compared our results in the normal vitreous body with those obtained in glaucomatous eyes.

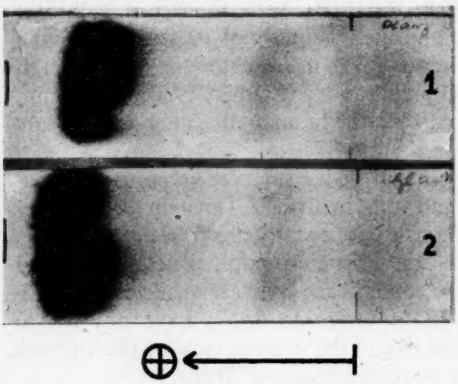

FIG. 1.—Paper electrophoresis (Cases 1 and 2).

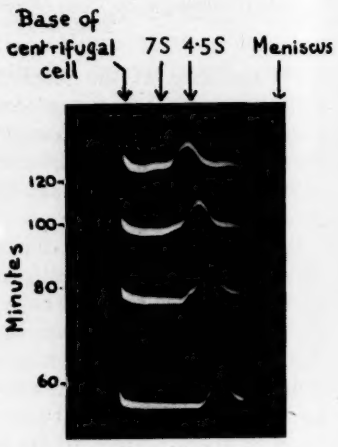

FIG. 2.—Composition of vitreous body proteins according to results of electrophoresis.

Methods

Vitreous body was investigated in freshly enucleated eyes. Immediately after operation the eyeball was opened by equatorial section, and the vitreous body carefully dissected out. This was diminished to about $\frac{1}{5}$–$\frac{1}{10}$ of its original volume through a 10 per cent. Dextran solution, and the concentrated solution so obtained was examined by paper electrophoresis (Fig. 1). In some cases further samples could be ultracentrifuged. The sedimentation was accomplished in a centrifugal field of 180,000g. and the 1·3 per cent. solution of human vitreous protein was examined in physiological saline without any addition (Fig. 2). In one case the ultra-violet spectrum of the protein solution was registered (Fig. 3).

The electrophoresis apparatus of Wunderly and others (1951) was used; the filter paper was Munktell (Sweden) type strips. The *p*H, buffer solution, and tension corresponded to the usual values for serum. The dried filter-strips were stained with bromine-phenol blue and the resulting coloured bands colorimetrically determined with the Beckman spectro-photometer after dilution of the dye. The electrophoresis diagram was prepared in the usual way (Fig. 4).

All the vitreous humours examined showed a good correlation with regard to their protein composition. Examples of four clinical cases are shown in the Table.

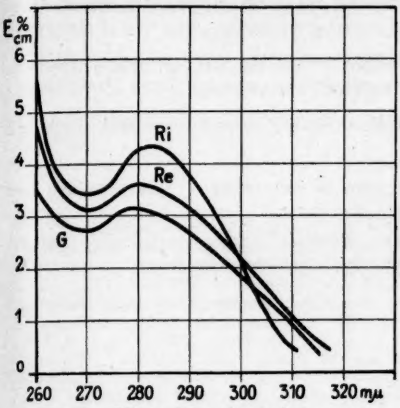

FIG. 3.—Ultra-violet absorption spectra.
G, vitreous humour.
Ri, lens protein.
Re, retro-retinal fluid.

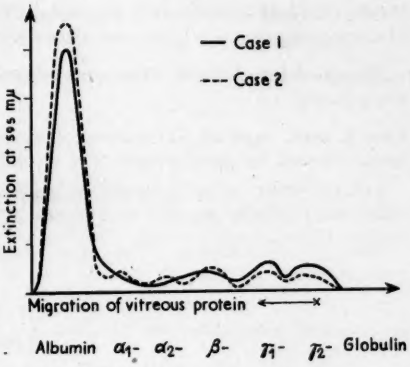

FIG. 4.—Electrophoresis diagram.

TABLE

PROTEIN COMPOSITION OF VITREOUS IN FOUR CASES

Case No.	Diagnosis	Albumin rel. per cent.	Globulin				
			α_1	α_2	β	γ_1	γ_2
1	Melanoma	61·0	7·6	5·4	10·4	7·6	8·0
2	Hydrophthalmos	77·8	3·8	5·4	7·8	5·6	4·3
3	Glaucoma	68·0	7·6	8·0	7·6	8·0	
4	Glaucoma	74·2	6·2	—	6·9	12·7	

Case Reports

Case 1, female, aged 70.—Ocular symptoms had first begun 4 weeks previously with the appearance of a shadow in front of the left eye.

Right eye normal both externally and ophthalmoscopically. Visual acuity 1.0.

Left eye showed no external irritation; anterior segment normal, lens and vitreous body clear. Tension 20 mm. Hg Schiötz, bullous detachment of retina with a tumour of the choroid below and nasally. Visual acuity 0.2.

Therapy.—Enucleation. The completely water-clear vitreous body of normal consistency was processed immediately.

Case 2, female, aged 32.—Congenital hydrophthalmos of both eyes. Recent episodes of increased pressure with pain.

Amaurosis. Both eyes staphylomatous with protrusion of the very thin sclera. Corneal diameter increased; large band-shaped corneal degenerations on both sides. Tension not constant, about 30 mm. Hg Schiötz.

Therapy.—Enucleation. The clear, somewhat fluid vitreous body was processed immediately.

Case 3, male, aged 52.—Chronic glaucoma in the left eye of several years' duration. For several days he had suffered intractable pain.

Left eye blind from chronic glaucoma. Tension 49 mm. Hg Schiötz. Early infection of anterior segment due to corneal ulcer. No response to treatment.

Therapy.—Enucleation. The clear, somewhat thready vitreous body was processed immediately.

Case 4, male, aged 68.—Occlusion of central vein of left retina 15 years before had been followed by development of a secondary glaucoma.

Left eye blind owing to secondary glaucoma. Tension 77 mm. Hg Schiötz. Painful infection of anterior segment with corneal dystrophy, resistant to treatment.

Therapy.—Enucleation. The clear, rather viscous vitreous body was processed immediately.

Results

The main portion of the human vitreous protein contains albumin, as demonstrated by electrophoresis and examination in the ultra-centrifuge. The fraction is homogenous and makes up 60–80 per cent. of the total protein. When the pressure is raised, this protein component seems to be increased, but any relationship with the duration or intensity of the increased pressure could not be demonstrated. It will be remembered that the colloid-osmotic pressure of a 1 per cent. solution of albumin amounts to 75 mm. H_2O, while that of a 1 per cent. solution of globulin amounts to only 19 mm. H_2O. (Wunderly and Wuhrmann, 1947). At the same time, the globulin subfractions are markedly diminished numerically, but the distribution within this non-homogenous group shows only a slight variation without specific characteristics. The most important result of this investigation is the demonstration that even under pathological conditions the proteins of the vitreous body contain no macroglobulins but only components of normal molecular size.

It would seem that the structural proteins of the human vitreous body consist mainly of a well-defined homogenous protein fraction similar to serum albumin. This interpretation is supported by the findings obtained by ultraviolet spectroscopy. The absorption of light in this spectral region depends for the most part on the content of tryptophane, tyrosine, and phenylalanine in the protein. The extinction co-efficients of these amino-acids are related as 27:8:1, although serum albumin contains only 0.1–0.2 per cent. tryptophane, while serum globulin contains 2–3 per cent. These important differences in chromophoric groups permit the characterization of a protein through the measurement of its specific absorption in the ultra-violet. The spectra of lens protein and retro-retinal fluid are illustrated for comparison (Fig. 4). The shape of the curve of the protein of the vitreous body, which shows a smaller absorption than lens protein and retro-retinal fluid and has its maximum at 280 mμ, corresponds to the large quantity of albumin contained therein.

The results of the analysis show that the protein fraction of the human vitreous body undergoes no important changes under conditions of increased pressure in the eye. The importance of the second structural component, hyaluronic acid, has not yet been ascertained.

We are indebted to the laboratories of the Hoffmann-La Roche Co., Basel, for the ultracentrifugal diagrams.

REFERENCES

BAURMANN, M. (1926). *v. Graefes Arch. Ophthal.*, **117**, 304.
CAGIANUT, B., and VERREY, F. (1949). *Ann. Oculist., Paris*, **182**, 649.
COMBERG, W. (1924). *Klin. Mbl. Augenheilk.*, **72**, 692.
DUKE-ELDER, S. (1930). "The Nature of the Vitreous Body". *British Journal of Ophthalmology*, Monograph Supplement No. 4.
FRIEDENWALD, J. S., and STIEHLER, R. D. (1935). *Arch. Ophthal., Chicago*, **14**, 789.
GRIGNOLO, A. (1952). *Ibid.*, **47**, 760.
HESSELVIK, L. (1939). *Skand. Arch. Physiol.*, **82**, 151.
LAUBER, H. (1936). *In* W. von Möllendorf, "Handbuch der mikroskopischen Anatomie des Menschen", vol. 3, pt. 2. Springer, Berlin.
MATOLTSY, A. G., GROSS, J., and GRIGNOLO, A. (1951). *Proc. Soc. exp. Biol., N.Y.*, **76**, 857.
MEYER, K., and PALMER, J. W. (1936). *J. biol. Chem.*, **114**, 689.
MÖRNER, C. T. (1893). *Hoppe-Seul. Z. physiol. Chem.*, **18**, 61.
PIRIE, A., SCHMIDT, G., and WATERS, J. W. (1948). *British Journal of Ophthalmology*, **32**, 321.
REDSLOB, E. (1932). "Le corps vitré". Masson, Paris.
SCHWARZ, W. Z. (1951). *Z. Zellforsch.*, **36**, 284.
—— and SCHUCHARDT, E., *Ibid.*, **35**, 293.
WUNDERLY, C., SCHNEIDER, G., and HUGENTOBLER, F. (1951). "C.R. 2e Congr. Int. Hémat., Rome", p. 240.
—— and WUHRMANN, F. (1947). *Schweiz. med. Wschr.*, **77**, 63.

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MERCURIALENTIS*

BY

DONALD HUNTER AND ARTHUR LISTER

London

Case Report

A MAN aged 58 had been employed for 35 years (1909–1939 and 1942–1947) as a thermometer maker. In 1935 the bulb of a long pipe thermometer containing 2 oz. mercury burst in the palm of his right hand and some of the mercury was deposited under the skin. Most of the mercury was removed at the time of the injury, but during the following week he developed ataxia and nystagmus and was thought to have acute labyrinthitis. He attended hospital regularly for 2 years, during which time his hand never healed completely. Finally he was admitted to hospital in 1937 when a grey scarred mass was excised from the palm of the right hand. During the following 2 months he developed symptoms of mercury poisoning with tremor of the arms and legs, ataxia, and severe headache. He became nervous and irritable, was never perfectly well from this time onward, and attended his doctor for many and varied complaints. In 1947 he had an attack of vomiting accompanied by vertigo and nystagmus which necessitated admission to hospital. Since this time he has complained of a staggering gait, of feeling miserable, and of continuous headache. Exposure to mercury ceased in March, 1948. When he was examined in January, 1951 there was no clinical evidence of chronic mercury poisoning. He was found to have vertigo, fine nystagmus, and a paresis of the right external rectus muscle. Both eyes had been trephined for chronic simple glaucoma.

The illustration shows the characteristic appearance in the lens on examination by the slit lamp, of a brownish, sharply-defined anterior band (*a* and *b*). It was the unusual definition of this band that first drew our attention to it. Examination with high power (*c*) showed it to be very finely granular and to have an iridescent sheen.

Atkinson (1943) examined seventy people who were or had been engaged in the manufacture of thermometers and found mercurialentis in 37, all of whom had been in close contact with mercury for from 3½ to 30 years though some had been out of contact with mercury for 10 to 20 years. Only fourteen of the seventy showed symptoms of chronic mercurialism, and all these showed mercurialentis.

Locket and Nazroo (1952) reported eye changes in 29 out of 51 men employed as direct current (DC) meter repairers. The DC meter consists essentially of an armature disk which revolves in a mercury bath between the two poles of a magnet. In the process of cleaning the meter, the armature disk is scraped in a lathe and particles of mercury in a finely divided form pass into the atmosphere. Twelve of the 29 men were found to have the brown reflex from the anterior capsule of the lens, ten had fine punctate lens

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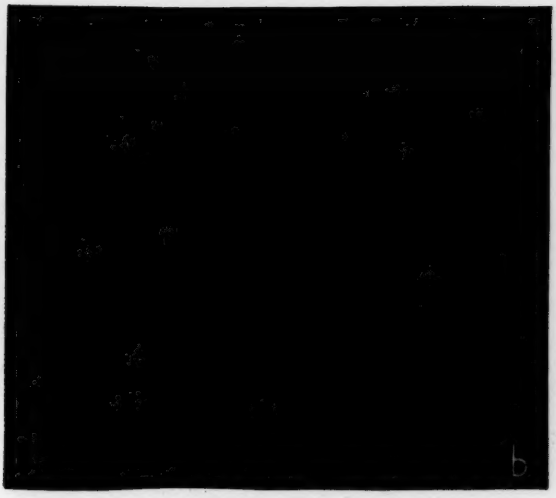

opacities, and 23 showed well-marked vascular changes at the corneoscleral junction. The brown reflex was only present in men with more than 5 years exposure to mercury.

It would seem therefore that mercurialentis is a common sign of long exposure to mercury; it may be the only sign and once formed is permanent. Although the condition is usually of industrial origin, it can also be caused by prolonged use of yellow mercury ointment on the skin and even on the lid margins (Rosen, 1950).

Our thanks are due to Miss Joy Trotman for the painting of the eye.

REFERENCES

ATKINSON, W. S. (1943). *Amer. J. Ophthal.*, **26**, 685.
ROSEN, E. (1950). *Ibid.*, **33**, 1287.
LOCKET, S., and NAZROO, I. A. (1952). *Lancet*, **1**, 528.

Brit. J. Ophthal. (1953) 37, 236.

TREATMENT OF *ESTABLISHED SYMBLEPHARON* WITH SPLIT SKIN HOMOGRAFT*

BY

G. J. ROMANES

From the Corneo-Plastic Unit and Eye Bank, Queen Victoria Hospital, East Grinstead, Sussex

THE condition of symblepharon is caused by the union of two opposing raw surfaces in the fornix between the lids and the globe of the eye following trauma or disease in the region. The traumatizing agents are most commonly thermal burns from boiling or exploding metal or chemical burns from lime and allied substances insufflated to the eye accidentally in the course of industrial processes.

The effects of the condition in the absence of corneal scarring are due to inadequate globe movements and are manifest to the patient as diplopia. More often the sight is impaired by corneal opacity and there is also some discomfort and watering of the eye and inability to move the lids or close them properly. The scar tissue also carries a vascular supply which runs on to the cornea and prevents effective keratoplasty without preliminary treatment.

The treatment of the condition is surgical and the methods used hitherto have not been altogether satisfactory. The aim is to divide the symblepharon and to prevent the reformation of scar tissue during the healing period. The new scar tissue, if allowed to develop, in time causes another symblepharon as extensive as its predecessor. The formation of scar tissue is prevented by the provision of cover for the raw area. In the past this has been done by local plastic procedures—a single pedicle conjunctival flap from above the limbus to the inferior fornix, where it is secured in position by sutures under tension. Alternatively, the defect was covered by a free graft of mucous membrane from the mouth, or by a split skin graft. The disadvantages of the first method are:

(*i*) There may not be sufficient conjunctiva available to provide all the cover required without suturing under tension.

(*ii*) If the flap is secured in this manner there is a great increase in the tendency to scar formation and to slow healing of the wound with its consequent disability.

(*iii*) The conjunctiva itself may be considerably scarred, so that there is also much shrinkage.

(*iv*) It may not be desirable for subsequent projected surgery to produce scarring all round the limbus.

* Received for publication November 11, 1952.

Mucous membrane grafts are bulky and unsightly. They can only be placed inside the lid and consequently their field of usefulness is limited.

Split skin grafts are rarely used in the presence of an eye because they shrink so extensively during the healing period. A socket which has been treated with skin grafts often has a great deal of discharge and desquamation which is a constant trouble to maintain under hygienic control.

It seemed possible that these difficulties would be overcome, at least in part, by using skin from another individual in the form of a free Thiersch graft to cover the bare area produced by the surgical manoeuvres. It is known that the skin does not survive for long so that it can cause none of the troubles mentioned above. When it sloughs off the process is gradual and there is no bare area exposed because the conjunctiva grows in to provide cover at the same rate as the exposure takes place.

Technique

The operation consists in incising the symblepharon at its attachment to the globe and separating it from the sclera and episcleral layers, from the limbus into the fornix. The globe is then rotated so as to produce as large a bare area as possible and to ensure that movement is perfectly free. The next step is to determine the size and shape of the defect. This is done by impressing upon it a piece of sterile Jaconet and obtaining a blood-stained " map ". This is used as a pattern to shape the graft of stored skin by cutting with scissors round the periphery of a stored-skin sample which has been spread upon Tulle Gras with the " map " superimposed upon it so that the nutrient surface of the graft will be facing towards the bare area. A graft of the exact shape and size of the defect is thus obtained. Unless this procedure is followed accurately the graft is of the wrong shape, and that makes the subsequent stage of suturing more difficult. The graft is then set into place with edge-to-edge apposition between it and the host epithelium all round its periphery, using 00 black silk interrupted sutures. The graft bed is washed free of clots using a lacrimal cannula on a syringe with normal saline. The patency of the fornix is maintained throughout the healing period by the insertion of a mattress suture over a short piece of No. 6 Jacques rubber catheter. The suture is tied out on to the cheek over rubber buttons to prevent possible pressure necrosis of the skin.

In the post-operative period both eyes are bandaged for 5 days to allow the graft to take. After this time the first dressing is done and the sutures removed; full movement of the eye is encouraged from then on.

We have always used general anaesthesia at this Unit but there is no contraindication to local infiltration and drops except the well-known difficulty of effectively anaesthetizing scar tissue. All donor skin is taken from patients with a known negative Wassermann reaction. The period of storage seems to be of little consequence; if the skin is stored for periods of 6 to 8 weeks in a refrigerator, a temperature of plus 4°C. is essential. The skin is wrapped in sterile damp gauze after being spread on tulle gras and kept in glass jars for use. For shorter periods a cool, sterile place is required, preferably a glass jar in a refrigerator. There is no contraindication to direct use, the skin being taken from one theatre and used almost immediately in an adjacent one. Skin has been used from donors ranging from 9 to 60 years of age.

Case Reports

(1) Aged 9 years.—*1947.* Hydrochloric acid burn with destruction of right globe retained disorganized (Fig. 1). Seen September, 1951.

Indication.—Socket preparation to retain artificial eye. Left concomitant convergent squint. Inadequate lower fornix. Movements of globe restricted.

3.9.51. Operation. Stored Thiersch skin 6 weeks old.

7.9.51. Complete take.

12.9.51. All skin in place, no stain with fluorescein (Fig. 2), no symblepharon, no skin visible, eye quiet, deep fornix (Fig. 3).

Operation. Squint correction, deep fornix as before (Fig. 4).

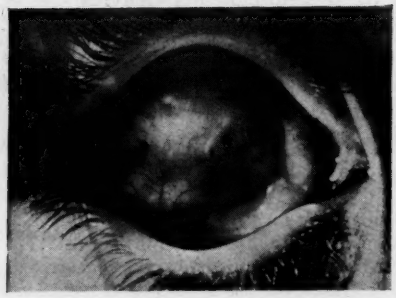

FIG. 1.—Case 1, as seen initially.

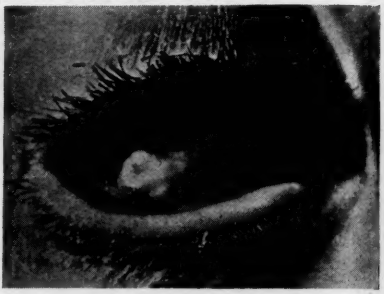

FIG. 2.—Case 1, homograft of skin in place and viable.

FIG. 3.—Case 1, fornix after disappearance of graft.

FIG. 4.—Case 1, artificial eye in place, lid pulled down to show lower fornix.

(2) Aged 24 years.—*1949.* Sulphuric acid burn left upper lid pars lacrimalis. Traumatic pterygium. Restricted movements (Fig. 5, opposite).

Indication.—Preparation for keratoplasty.

11.12.51. Operation.

17.12.51. Stitches out, movements full; skin viable beyond margin of raw area, deep fornix (Fig. 6, opposite).

31.12.51. No skin remaining, full movements of globe, no stain with fluorescein.

24.3.52. Full movements of globe, normal fornix (Fig. 7, opposite).

(3) Aged 18 years.—*1950.* Metal burn in left eye September 7 (Fig. 8, opposite).

Indication.—Preparation for keratoplasty.

8.1.51. Division of symblepharon and mucous membrane graft; fornix depth unchanged with some ectropion.

FIG. 5.—Case 2, as seen initially.

FIG. 6.—Case 2, homograft of skin in place and viable.

FIG. 7.—Case 2, end-result of symblepharon repair before keratoplasty

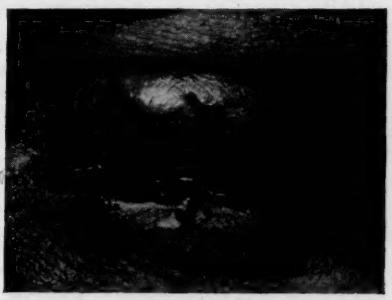

FIG. 8.—Case 3, as seen initially.

FIG. 9.—Case 3, homograft of skin in place and viable.

FIG. 10.—Case 3, end-result of symblepharon repair before keratoplasty.

5.3.51. Correction of ectropion of graft by conjunctival plastic. Result: no depth to fornix.

23.5.51. Operation. Division of symblepharon and insertion of contact glass. Result: no depth to fornix.

1.10.51. Division of symblepharon stored skin graft. Result: good fornix, still old ectropion. (Fig. 9).

5.5.52. No skin left. Eye healed. Fornix deep. Movements of globe full (Fig. 10).

(4) Aged 68 years.—Solder exploded 47 years ago.
Indication.—Preparation for keratoplasty.
12.2.52. Operation. Division of symblepharon L. Stored skin inserted over contact glass.
24.2.52. Skin alive and healthy, good deep fornix.
30.5.52. All skin gone, fornix satisfactory.

(5) Aged 39 years.—*1949.* Incendiary bomb incident.
Indication.—Preparation for keratoplasty. Seen August, 1951, through stages of final repair.
February 1952. Operation. Traumatic pterygium treated MacReynolds, corneal bare area covered by homograft.
27.2.52. Corneal cover satisfactory.
11.7.52. No sign of graft, movements of globe free, awaiting keratoplasty.

(6) Aged 27 years.—*April 1950.* Metal burn right eye; treated with amnion graft; result symblepharon.
October 1950. Mucous membrane graft to lower fornix: result, shallow fornix, and graft overlying cornea (Fig. 11).
Indication.—Preparation for keratoplasty.
7.3.51. Seen as out-patient.
7.4.52. Operation, excision of mucous membrane graft, refashioning of inferior fornix and grafting with split skin homograft.
14.4.52. Discharged home with skin viable and in place.
12.5.52. See as out-patient, no skin left, fornix satisfactory.
22.8.52. Movements of eye full, fornix satisfactory (Fig. 12).

FIG. 11.—Case 6, as seen initially.

FIG. 12.—Case 6, end-result of symblepharon repair before keratoplasty. Note eversion possible to show fornix depth.

(7) Aged 52 years.—Lime burn both eyes at age of 6 years. Not seen until *28.3.52.*
Indication.—Preparation for keratoplasty.
29.7.52. Operation, division of symblepharon and grafting with split skin homograft.
12.9.52. Inferior fornix free, movements of eye full.

(8) Aged 6 years.—*May 1951.* Lime burn of right eye. Treated with amniotic membrane graft, result, extensive symblepharon and corneal destruction.
26.6.52. Seen total symblepharon of lower lid on right side.
Indication.—Preparation for retention of prosthesis.
13.9.52. Operation, division of symblepharon and grafting with split skin homograft.
5.10.52. Complete take of graft.
11.10.52. Skin starting to slough, movements of globe satisfactory, fornix depth satisfactory, discharged home.

(9) Aged 29 years.—*May 1949.* Bilateral lime burns, left eye enucleated because of total destruction. Right eye showed gross destruction of cornea and conjunctiva.
Indication.—Preparation for keratoplasty.

March, 1952. Seen, almost total symblepharon of upper lid with corneal destruction and infiltration with vessels.
15.7.52. Operation, division of symblepharon and grafting with homograft.
22.7.52. Full movements, graft in place.
29.9.52. Seen as out-patient, no skin in place, fornix satisfactory, movements of eye full.

(10) Aged 53 years.—*January 1952.* Molten metal burn of both eyes.
Indication.—Preparation for keratoplasty.
10.6.52. Operation, division of symblepharon and homograft to defect.
27.6.52. Homograft being shed.
11.7.52. No sign of homograft, inferior fornix deep.
26.9.52. Homo skin graft to upper fornix after symblepharon division.
5.10.52. All graft shed, eye quiet, movements good, superior fornix deep, awaiting keratoplasty.

The use of homografts which survive 2 to 3 weeks thus overcomes many difficulties encountered hitherto in the surgery of established symblepharon. The graft is of the right thickness and is easily obtained in adequate quantity. There is no ultimate discharge and no long-term cosmetic factor to be considered in planning the operation. The graft can be set, therefore, upon the best functional site. It is easy to manipulate at operation and is of satisfactory origin. The grafted eyes are quiet in the post-operative and subsequent period. The final healing in the cases treated is completed in a short time and is associated with the minimum of scar-tissue formation. This is due to the very rapid proliferation of the conjunctiva which, combined with the small size of the site, keeps the surface of the whole bare area covered during the necrotic stage. Any scar tissue which forms lies upon the sclera or episclera which is quite able to withstand the shrinking stages. This position also allows for full movement of the lid and obviates all tendency to loss of fornix depth.

It is probable that the repair described is unusual in surgery, since reference to the literature on homografts shows that hitherto the authors have all been concerned to discover and eliminate the factors which lead to the death of the graft in order to obtain permanent and stable survival.

Summary

A satisfactory method of surgical repair of traumatic symblepharon using stored homografts of skin is described in ten cases and photographs.

I wish to acknowledge with gratitude the help I have had in the preparation of this paper from Mr. B. W. Rycroft, Surgeon to the Corneo-Plastic Unit. I am indebted to Mr. L. Schofield for much information on homograft behaviour and to him and colleagues of the surgical staff of the Plastic and Jaw Unit for the provision of skin without which the method could not have been tried. I also wish to thank Mr. Gordon Clemetson for the photographs.

REFERENCE

SPAETH, E. B. (1948). "Principles and Practice of Ophthalmic Surgery", 4th ed., p. 529. Kimpton, London.

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OCULAR FINDINGS IN HAEMOCHROMATOSIS*

BY

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HAEMOCHROMATOSIS is a rare condition, the chief clinical features of which are pigmentation of the skin, cirrhosis of the liver, diabetes mellitus, and hypogonadism. The opportunity of examining the eyes of five patients in whom the diagnosis had been confirmed by liver biopsy arose through the kindness of my colleague, Dr. N. F. Coghill, who is incorporating the clinical and pathological investigations in a forthcoming publication. Detailed clinical histories have not, therefore, been included in the present communication.

A review of the literature since 1930 has revealed very few references to the ocular findings. Maddox (1933) describes the retinal appearances observed in four cases, and this reference is quoted by Duke-Elder (1940). Lawrence (1949), in his Banting Memorial lecture, commented upon the absence of vascular changes in the pure and primary forms of islet and pancreatic destruction, and contrasted these with appearances found in primary diabetes; he suggested that a common factor, perhaps the pituitary, might be responsible for both the vascular degeneration and the diabetes, and then referred to two case-reports (relapsing pancreatitis—Sprague, 1947; haemochromatosis—Stawell, personal communication), in which the retinal changes of diabetes were observed.

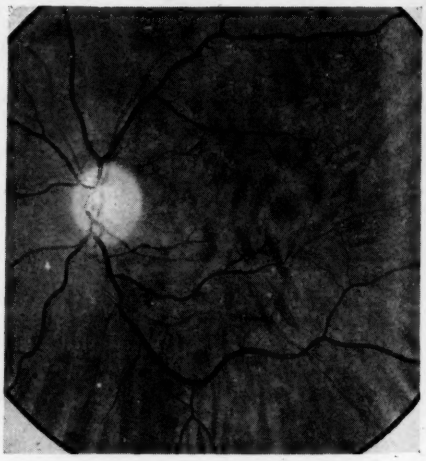

FIG. 1.—Left fundus of Case 2.

Clinical Findings

There is to date no record in the literature (apart from Lawrence's mention of Stawell's case), of fundus changes similar to those of diabetes mellitus occurring in patients with primary destruction of pancreatic tissue. Changes of this type have been observed (Fig. 1) in one patient (Case 2) of the present series. The ocular findings in all five cases are described in detail below, and a summary of the appearances in individuals is given in Table I.

* Received for publication November 11, 1952.

TABLE I

OCULAR APPEARANCES IN FIVE CASES OF HAEMOCHROMATOSIS

Case No.		1	2	3	4	5
Conjunctiva ...		Occasional fusiform aneurysms	Fusiform and "berry" aneurysms	Fusiform and "berry" aneurysms	Fusiform and "berry" aneurysms	Normal
Cornea		Normal (arcus senilis)	Normal	Normal	Normal	Normal
Iris		Brownish-green	Brownish - green	Brownish - green	Brownish - green Melanoma of left iris	Brownish - green
Lens		Clear	Few vacuoles Relief of adult nucleus	Fine white dot opacities in nucleus, R<L	Clear	Few vacuoles in right lens
Vitreous ...		Normal	Normal	Normal	Normal	Normal
Fundus... ...		Normal	Early diabetic and arteriosclerotic changes (Figs 1 and 2).	Normal	Normal	Irregular reflex at right macula
Visual Acuity	R	+2.50/−1.75 × 95° = 6/5	−2.00/+1.25 × 80° = 6/5	+4.00/+0.75 × 165° = 6/6	−0.25/+0.75 × 95° = 6/5	+0.50 = 6/5
	L	+2.75/ - 1.35 × 85° = 6/5	−1.25/−1.25 × 95° = 6/5	+3.75/+0.75 × 180° = 6/6	−1.00/+2.25 × 40° = 6/6	+0.50/+0.50 × 110° = 6/5

Conjunctiva.—The conjunctival vessels showed both fusiform and saccular aneurysmal dilatations in three cases, fusiform aneurysms in one, and no micro-aneurysms in the fifth. The changes were found particularly in the superficial vessels within about 5 mm. of the limbus (Fig. 2; see also Fig. 3 overleaf). The relationship between diabetes and the presence of aneurysms is given in Table II (below). Only two patients were suffering from diabetes mellitus.

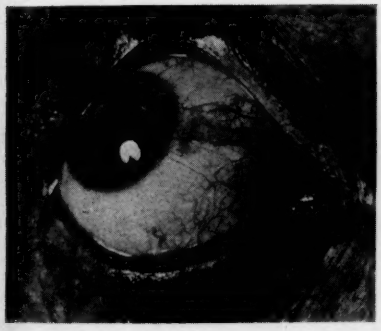

FIG. 2.—Conjunctival aneurysms in Case 4.

Cornea.—No characteristic changes seen.

Iris.—The irides in this group were all of a similar brownish-green colour, but no significance is attached to this. The microscopic appearances in one case are recorded below.

TABLE II

RELATIONSHIP BETWEEN DIABETES AND MICRO-ANEURYSMS

Case No.	Age (yrs)	Duration of Illness (yrs)	Micro-aneurysms		Glucose Tolerance Curves
			Conjunctival	Retinal	
1	63	7	+	−	"Lag" type
2	50	15	++	+	Diabetic
3	50	2	++	−	"Lag" type
4	66	2	++	−	Diabetic
5	45	6	−	−	Normal

Lens.—The appearances varied in individual patients; the lens changes described in Table I are those which would be expected in a group of normal subjects of the same age.

Vitreous.—No characteristic changes were observed.

Retina.—In Case 2 (Fig. 1) the fundi showed changes consisting of a few micro-aneurysms of the " berry " type along the course of the main retinal vessels in the right eye between the disc and the equator, a few micro-aneurysms along the main vessels, and two small patches of retinal haemorrhage along the course of the inferior temporal artery in the left eye. The fundus ground around the disc appeared to be rather more deeply pigmented than in a normal subject, but this was not a gross change.

FIG. 3.—Conjunctival aneurysms in Case 4 (high-power magnification).

One patient (Case 4) was found on examination to have a melanoma of the left iris, and he subsequently attended Moorfields for further opinions as to the possible malignancy of the condition. As the majority of the opinions favoured a malignant aetiology, it was decided to perform a complete iridectomy, with a fairly wide margin of normal tissue around the tumour. From the point of view of the present paper, it is of interest that no free iron pigment was detected in any of the serial sections. The full pathological report on Case 4 states:

Sections show a highly cellular benign pigmented naevus of the iris. The lesion is surrounded on both sides with normal tissue and has, therefore, probably been completely removed. No free iron pigment was detected in any of the serial sections.

Discussion

The presence of conjunctival aneurysms has been observed in four of the five cases, two being truly diabetic, and two showing a " lag " type of glucose

tolerance curve. With the possible exception of the case in which the fundus around the disc appeared unduly dark, no changes characteristic of haemochromatosis have been observed. The most obvious fundus changes were those which could be attributed to diabetes. The absence of free iron pigment in Case 4, in whom an iridectomy was performed for a suspected malignant melanoma, supports the view that free iron pigment is probably not deposited in uveal tissue. With one possible exception, the primary change referred to by Maddox (1933) has not been observed in any of the cases described. The exact pathology of the vascular lesions must remain a matter for discussion until pathological material has been examined.

The ocular changes in diabetes have been described several times, and attention has recently been drawn to the presence of retinal micro-aneurysms in " normal " eyes, and in many diseases other than diabetes (Ashton, 1951). The significance of the vascular changes observed clinically in one of the present series of cases of haemochromatosis is, therefore, a matter for speculation, and it is not possible to say more than that the fundus changes are *similar* to those found in diabetes.

McCulloch and Pashby (1950) have examined the conjunctival vessels in normal subjects, and in two groups of diabetic patients; one group with an associated diabetic retinopathy, and the other group without. They found conjunctival aneurysms in 55 per cent. of diabetic and 14 per cent. of non-diabetic patients, the aneurysms being of the " berry " type described by Ballantyne and Loewenstein (1943) in their observations on the vascular changes in the fundus. No comment is made on the glucose tolerance curves in the " non-diabetic " group. Ashton (1949) reported the *post-mortem* findings in 21 diabetics, and in these cases conjunctival aneurysms were not observed. Their absence in normal subjects is supported by the work of Dobree (1950), who describes the perilimbal vessels in the normal and congested eye, and does not mention the presence of aneurysmal dilatations of either of the types described by Ballantyne and Loewenstein. In view of the inconclusive evidence so far presented in the literature, it is proposed further to investigate the relationship between conjunctival micro-aneurysms and diabetic conditions, and the incidence of these vascular anomalies in normal subjects.

The observations recorded above give no indication of the occurrence of ocular changes characteristic of haemochromatosis. The appearances are similar to those found in diabetes, which occur in about three-quarters of patients with the condition.

Summary

The ocular findings in five cases of haemochromatosis are reported. Conjunctival micro-aneurysms and fundus changes similar to those of early

diabetic retinopathy are recorded. No other ocular findings characteristic of haemochromatosis have been observed. The significance of the findings is discussed.

REFERENCES

ASHTON, N. (1949). *British Journal of Ophthalmology*, **33**, 407.
——— (1951). *Ibid.*, **35**, 189.
BALLANTYNE, A. J., and LOEWENSTEIN, A. (1943). *Trans. ophthal. Soc. U.K.*, **63**, 95.
DOBREE, J. H. (1950). *British Journal of Ophthalmology*, **34**, 720.
DUKE-ELDER, S. (1940). "Text-book of Ophthalmology," vol. 3, p. 2744. Kimpton, London.
MADDOX, K. (1933). *British Journal of Ophthalmology*, **17**, 393.
MCCULLOCH, C., and PASHBY, T. J. (1950). *Ibid.*, **34**, 495.
LAWRENCE, R. D. (1949). *Lancet*, **2**, 401.
SPRAGUE, R. G., (1947). *Proc. Mayo Clin.*, **22**, 553.

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SARCOIDOSIS OF THE EYE*†

BY

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SARCOIDOSIS (*Boeck's sarcoid; Besnier-Boeck disease;* benign lymphogranulomatosis; uveo-parotid fever; Heerfordt's disease) has been defined as:

> A disease in which the lesions have a characteristic histological appearance consisting of epithelioid cell tubercles, as a rule clearly defined within the affected tissue, with only slight round cell infiltration in their vicinity, without central caseation, with or without Langhan's type giant cells, and with or without peculiar inclusion bodies within these cells, proceeding in the older lesions to conversion into hyaline fibrous tissue; these histological appearances must be present in all affected tissues. (Robb-Smith, 1952).

Pathology.—The structural changes which may commonly affect the skin, bones, lymph glands, lungs, and eyes, and rarely almost any tissue of the body, result from the formation of small nodules, composed of epithelioid cells, which never show necrosis. Some lymphocytes may be present around the nodules, and a few giant cells may be found which contain small refractile bodies. Healing follows by organization and fibrosis, but calcification does not occur.

General Signs.—Skin nodules and enlargement of the lymph glands are commonly seen, *X*-ray examination of the bones shows characteristic rarefaction, especially in the phalanges, and radiological examination of the chest shows the diffuse fibrosis of the lungs and enlargements of the mediastinal lymph glands which is present in nearly all cases of the condition. The parotid salivary gland may be enlarged, and palsy of the facial nerve may be a sequel. The submaxillary salivary gland and the lacrimal gland are also affected in some patients giving the syndrome of von Mickulicz. Nodular lesions may occur more rarely, in almost all the tissues of the body. Laboratory investigation often reveals an increase of the erythrocyte sedimentation rate, and an elevation of the serum globulin. A very common finding, and one which some authorities suggest is present in all cases of sarcoidosis, is an anergy to tuberculin. No skin reaction occurs as a result even of large intracutaneous doses of the tuberculin, and the injection of B.C.G. vaccine does not induce a positive skin reaction to tuberculin in most cases. In Great Britain, therefore, where almost all adults show a positive Mantoux test, a negative reaction should always lead to the suspicion that sarcoidosis may be present.

* Received for publication December 10, 1952.

† Dedicated to Professor Dr. Karl Lindner on the occasion of his 70th birthday.

Ocular Signs.—Sarcoidosis may occur in any part of the eye or ocular adnexa, but the uveal tract is the part most frequently affected. Nodules appear in the iris which may when fully formed, be large and more vascular than those of tuberculosis (Laval, 1952). Large clear keratic precipitates are present, often situated peripherally, and posterior synechiae may occur. The inflammatory reaction may be acute, subacute, or chronic. Choroidal lesions occur rarely, and the retina may be involved; lesions of the latter structure are sometimes accompanied by periphlebitis. Swelling of the parotid gland in association with uveitis, fever, and general malaise is the syndrome of uveoparotid fever.

Aetiology.—The essential feature of sarcoidosis is a hyperplasia of mesenchymal cells to form histiocytes and epithelioid cells. Present evidence suggests that this can only occur as a response to infection by, or sensitivity to, members of the mycobacterium group. The difference between tuberculosis and sarcoidosis seems small in some cases (Merz, 1952). The granulomata which form in response to beryllium appear, according to some authorities, to have different histological characteristics, and it is stated that this lesion must be regarded as a separate condition.

Diagnosis.—The clinical characteristics, the radiological appearances, the anergy to tuberculin, and the rise of serum globulin, indicate the nature of the condition, though the final test should, where possible, be histological examination of one of the lesions either of the skin or of a lymph gland. It is possible that some cases cause symptoms and signs only in the eye, and that these may be regarded, in error, as cases of non-specific uveitis.

Case Reports

(1) Aged 20.—Admitted to the Eye Sanatorium, Swanley, on August 2, 1951, with a history of painless congestion of the left eye with blurred vision for 4 months. The right eye was normal. The left eye, in which vision was hand movements, showed a ciliary staphyloma near the limbus above, a large iris nodule, and large pale keratic precipitates. The Mantoux reaction was negative to 1:100 O.T. and remained so even after B.C.G. vaccine. Cervical, axillary, and mediastinal lymph glands were enlarged, and biopsy showed the typical histological changes of sarcoidosis. In October, 1952, the condition of the left eye started to settle, coinciding with the beginning of a prolonged course of subconjunctival injections of cortisone. The iris nodule had disappeared from the left eye on December 9, 1952, and visual acuity was 6/36.

(2) Aged 58.—Admitted to the Eye Sanatorium, Swanley, on June 16, 1951, with a history of left uveitis of 3 months' duration. There was dryness of the mouth, and both eyes showed evidence of keratitis sicca. There was no evidence of uveitis in the right eye, but the left showed profuse keratic precipitates. No iris nodule was seen. The Mantoux reaction was negative to 1:100 O.T. and remained so after injection of B.C.G. vaccine. Radiological examination of the chest showed diffuse reticulation of the lungs, an appearance compatible with sarcoidosis. The condition of the left eye improved markedly after a period of general rest, and local treatment to the eye with atropine drops.

(3) Aged 26.—Admitted to the Eye Sanatorium, Swanley on May 23, 1952, with

a history of recurrent uveitis of 3 years' duration in both eyes, and of scarring of one lung, visible on radiological examination. Both eyes showed a mass of pale keratic precipitates. The Mantoux reaction was negative to 1:100 O.T. and some enlarged cervical glands were found to be present. This patient discharged herself from hospital before treatment could be completed.

(4) Aged 30.—Admitted to the Eye Sanatorium, Swanley, on December 17, 1951, with a history of recurrent uveitis of 10 years' duration in both eyes, and of an attack of pneumonia 9 years before. Both eyes showed old keratic precipitates and a few small nodules were present in the right iris. Posterior cortical lens opacities were present in both eyes. The Mantoux reaction was negative to 1:100 O.T. but became positive to the same dose of O.T. after an injection of B.C.G. vaccine. No change in the ocular condition followed 3 months' treatment in hospital, and the condition remains unsettled.

(5) Aged 28.—Admitted to the Eye Sanatorium, Swanley, on July 2, 1951, with a history of bilateral uveitis of 2 years' duration. Pale keratic precipitates were present in both eyes. The Mantoux reaction was negative to 1:100 O.T. but became positive to the same dose after an injection of B.C.G. vaccine. Radiological examination of the chest showed lesions compatible with mild sarcoidosis. The eye condition improved slightly during 4 months' stay in hospital.

(6) Aged 38.—Admitted to the Eye Sanatorium, Swanley, on July 30, 1951, with a history of bilateral uveitis of 3 years' duration. Large clear keratic precipitates were present in both eyes. The Mantoux reaction was negative to 1:100 O.T. but became positive after an injection of B.C.G. vaccine. Radiological examination of the chest showed lung changes suggestive of sarcoidosis. There was improvement both of the ocular and general condition during a stay of 3 months in hospital.

(7) Aged 23.—Admitted to the Eye Sanatorium, Swanley, on June 6, 1951, with a history of recurrent uveitis in both eyes of 4 years' duration, and of iris nodules present in the left eye for one year. Both eyes showed old keratic precipitates and some nodules were present in the left iris. The Mantoux reaction was negative to 1:1000 O.T. Radiological examination of the hands showed changes suggestive of sarcoidosis in the phalanges. Considerable improvement in ocular condition occurred during 4 months' stay in hospital.

Discussion

Sarcoidosis must be regarded as a tissue reaction to a foreign agent which is introduced into the body. This is possibly a hypersensitivity reaction of certain cells of the body analogous to that which is believed to occur in uveitis, or it is possibly a direct reaction to a toxic agent. One body of opinion believes that this sensitizing or toxic agent is related to the mycobacterium group, but in this series of cases, little evidence of tuberculous infection was found. In Great Britain, however, the Mantoux reaction is positive in almost all adult persons, and the possibility of a subclinical tuberculous infection in these cases of sarcoidosis cannot be excluded. Typical cases of sarcoidosis show characteristic changes in the lymph glands, lungs, and eyes, as well as in other parts of the body, but consideration must be given to cases showing less typical and less widespread signs. No cause is found for many cases of uveitis, and in the presence of a negative Mantoux reaction the possibility of sarcoidosis should always be considered. The diagnosis is confirmed by the presence of enlarged lymph glands, shadowing of the lungs on radiological examination, and a raised serum globulin, as

well as by the development of iris nodules, and by the large transparent peripheral keratic precipitates which are found so frequently in this condition. Absolute proof depends upon the histological appearances of an excised lesion.

Tuberculin anergy in sarcoidosis is a constant finding. It is due to the presence in the skin of a substance which has been called anticutin and which prevents a hypersensitivity reaction to injections of tuberculin. Anticutin remains in the skin even when its hypersensitivity is increased by the injection of B.C.G. vaccine.

The cause of sarcoidosis is uncertain and treatment cannot, therefore, be based on logical principles. Cases treated at the Eye Sanatorium, Swanley, on a regime of general rest have improved, though since the disease seems to be chronic in many cases, complete cure does not occur in the 3–6 months which they spend as patients. The use of cortisone appears to have been beneficial in some cases, and this would support the thesis that hypersensitivity is a cause of the condition. ACTH may also be useful (Ozazewski and Bennett, 1952). In some cases the condition may persist for many months or even years, but the ultimate prognosis for vision appears to be good.

REFERENCES

LAVAL, J. (1952). *Amer. J. Ophthal.*, **35**, 551.
MERZ, M. (1952). *Klin. Oczna*, **22**, 49.
OZAZEWSKI, J. C., and BENNETT, V. (1952). *Amer. J. Ophthal.*, **35**, 547.
ROBB-SMITH, A. H. T. (1932). *In* " Modern Practice in Tuberculosis ", ed. T. Holmes Sellors and J. L. Livingstone, vol. 2, p. 262. Butterworth, London.

Brit. J. Ophthal. (1953) **37**, 251.

APPLIANCE

TABLE VIEWER AS AN AID TO CLINICAL DEMONSTRATION*†

BY

JOHN FOSTER

Leeds

WHILE a dermatologist can demonstrate a case to a dozen students simultaneously, the ophthalmologist, owing to the size or position of eye lesions, may have to do so to the members of his class successively. To avoid delay and discomfort to patients, it is essential, therefore, that each student should understand clearly in advance what he is trying to see.

At Edinburgh, lantern-lectures are given in a theatre in the clinic itself, the subject being determined by the presence of one or two cases of a given disease, which are thereafter demonstrated clinically. This has the added advantage that pictures can be shown of other stages and of the underlying pathology of the disease. Facts demonstrated so systematically are far more likely to be retained then the salient points of six or seven cases demonstrated clinically as they occur in out-patients.

Unfortunately, in the Leeds Infirmary Eye Department, as in most teaching hospitals, there is not enough space for a conventionally annexed lecture-theatre for lantern-slide projection.

Apparatus

A compromise solution occupying little space may be found in the Kodaslide Table Viewer. This projects 35-mm. slides on to a small ground glass screen (8″×8″) which is visible in daylight to six or twelve people at once. As they could not obtain an import licence for this instrument, which is made in the U.S.A., Messrs. Theodore Hamblin and Co. made the device shown here. It consists of an Aldis projector, which is British-made and available in most teaching hospitals, fitted in a collapsible box containing a mirror which reflects the projected image on a ground glass daylight viewing screen. The projector and a plastic case for fifty slides fit into the box; the dimensions are 21″ long, 14″ wide, and 11″ high when folded up, and 38″ long, 14″ wide, and 19″ high when fully extended (see Figure overleaf).

Slides for Elementary Teaching.—Hamblin's catalogue contains 337 coloured slides of ophthalmological conditions, but these are hand-painted and necessarily expensive.

After some experiment the firm have been able to reproduce these as 35-mm. transparencies suitable for this instrument at one-eighth of the price. The colour values, while naturally not as perfect as the originals, are adequate for undergraduate and even post-graduate teaching.

* Received for publication January 6, 1953.

† Read to the North of England Ophthalmological Society on November 29, 1952.

Pictures of a certain number of common conditions (*e.g.*, iridocyclitis, fluorescein-staining, corneal ulcer, lacrimal abscess, and ophthalmia neonatorum) were not available and these have had to be synthesized with certain features a " little larger than life ". It may be possible later on to replace some of these by colour photos as suitable cases present themselves.

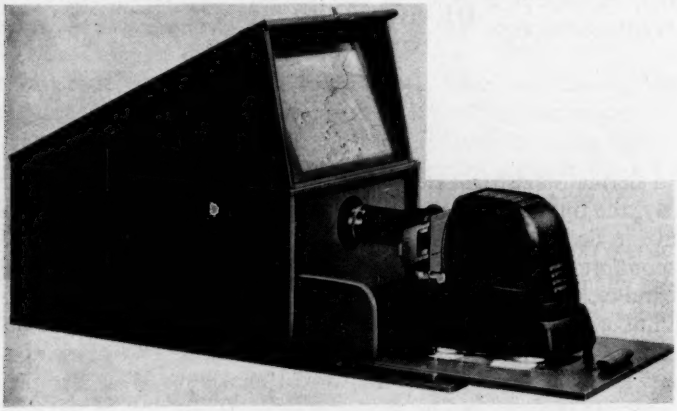

FIGURE.—Table viewer for use with coloured slides.

The selected series of fifty slides covers the diagnostic aspect of elementary clinical ophthalmology fairly well, and this can even be done by a smaller number if certain " comparative slides " devised by the author are employed.

These slides are fundus pictures, divided into quadrants, each representing a separate fundus condition. For example the differences due to pigmentation in the appearance of the normal fundus in blonde, brunette, Mediterranean, and negroid types.

The undergraduate of today does not seem to have enough time to attend the special operating theatres, so that for clinical (and even more for didactic lecturing) slides illustrating eye operations would also be of value. A further series of fifteen to twenty semi-diagrammatic slides in black and white to cover this part of the subject is in preparation.

CORRESPONDENCE

STANDARDIZATION OF READING TYPES

To the Editorial Committee of the BRITISH JOURNAL OF OPHTHALMOLOGY

DEAR SIRS—I read with interest and approbation the preliminary memorandum on the Standardization of Reading Types presented by Mr. Frank Law for the consideration of the Council of the Faculty and for comment from the profession at large (*British Journal of Ophthalmology*, 1951, **35**, 765). In a recent issue (*British Journal of Ophthalmology*, 1952, **36**, 689) Mr. Law has set forth the final recommendations of the Council on this subject. I realize therefore that it is too late for effective intervention. There is one point, however, in this second publication which calls for comment as it has an important bearing on our everyday practice and concerns the application of these proposed reading types. When explaining the reduced Snellen type Mr. Law says: " If this type be reduced to 1/17 of its normal size, the letters in the 6/6 line subtend 5 minutes at a distance which very closely approximates to 35 cm. or 14 in., *which is an average reading distance.*" (my italics).

Surely I am not alone in asserting that this is an important misconception: a misconception which I know used to be not uncommon, but which I had ventured to assume would not be perpetuated by being given the authority of the Faculty.

I usually measure the distance between the eyes and the type unconsciously chosen by the patient before any attempt is made to select a passage for reading. Also I have made a practice (and who has not?) of observing the reading and working habits of my fellows in conditions outside the consulting room. Although it might be argued that the majority are in dire need of a new prescription, nevertheless I am satisfied that the average reading distance is certainly not less than 40 cm. or 16 in. A very large proportion prefer 18–22 in., whilst a small minority, mostly women given to sewing, prefer 14 in. Fortunately it is only in those cases where there is no residue of accommodative power when one has to be precise in choosing an exact reading addition, and I would usually choose a plus 2.50 addition for a pair of 6/5 aphakic eyes, sometimes plus 2.25 or plus 2.0, but only rarely plus 2.75 or plus 3.0, one of which would have to be chosen if the average reading distance were genuinely 14 in.

In my opinion the conception of a 14-in. average distance can only tend to over-prescribing and a no doubt welcome increase in the orthoptist's clientèle.

Furthermore, although the reduced Snellen type is to be used for "an accurate relative assessment for comparative purposes ", the " point " types need not be made unnecessarily valueless in assessment of the vast majority of cases by using the smallest type (5 point) at 14 in. The variation in acuity capable of reading this type at this distance is considerable (6/4 to 6/12); at 16 in. it would be more selective and at 18 in. still more so. I myself can read the 5-point example (*British Journal of Ophthalmology*, 1951, **35**, 772) with each eye separately, as far away as 65 cm. or nearly 26 in. and I make no claim to possessing supra-average eyes.

Yours faithfully,

E. G. RECORDON

51, BATEMAN STREET,
CAMBRIDGE.

To the Editorial Committee of the BRITISH JOURNAL OF OPHTHALMOLOGY

DEAR SIRS—May I reply to Mr. Recordon's letter. His objection has substance but I believe arises through slovenly expression on my part. I think that what I and the Faculty Council meant was that reading glasses should enable a patient to read at 35 cm.—for there are many conditions under which this is necessary. Except in the very old, or in paretic cases, the range afforded is considerable, and the patient is by no means restricted to this distance by a properly prescribed pair of readers.

Regarding the " Snellen Equivalent ", it is important to note the contents of the third paragraph of the second article on Reading Types (*British Journal of Ophthalmology*, 1952, **36**, 689), where it is clearly pointed out that the reduced Snellen type is not to be used in the ordinary sense of a test, but as a means of recording the near acuity on a scientific basis. For this purpose clearly an arbitrary distance must be specified, and this has been done. To what extent this record (when it is made) shall influence the strength of reading glasses eventually prescribed is entirely in the hands of the examiner.

Yours faithfully,

FRANK W. LAW.

36, DEVONSHIRE PLACE,

LONDON, W.1.

January 30, 1953.

BOOK REVIEW

Ophthalmic Plastic Surgery. By SIDNEY A. FOX. 1952. Pp. 290, 133 figs. Bibl. Grune and Stratton, New York. (£6).

The author has drawn extensively on his own large experience in plastic surgery of the eyelids and orbit, and he describes those operations which he has found to work well in his hands. His descriptions of operations are well illustrated, some by photographs but mainly by line and wash drawings which show clearly the steps of the reconstructive work. After the account of almost every operation, there is a commentary on alternative procedures and the reason for the choice of the operation described.

The author might with advantage have stressed the urgency of providing skin cover when all four eyelids are severely burnt in war and aviation injuries. He begins Chapter IV on technical details with the unfortunate comment " A chapter on this subject is usually written to be ignored ". Technique is of immense importance in reconstructive surgery, and it would have been well if the author could have instructed his ophthalmologist readers by a fuller description of surgical technique and the necessary instruments to achieve this.

There are several small omissions, in particular the need for suturing the orbicularis oculi as well as the periosteum over a cartilage wedge graft in the floor of the orbit; and the suture of the bulbar and palpebral conjunctiva after division of a small symblepharon by a vertical opposed to a horizontal line.

Few plastic surgeons would agree to the closure of a large lower-lid coloboma by a sliding advancement flap brought up from the cheek between two vertical incisions. Secondary scars placed at right angles to normal lines of tension and the downward traction of subcutaneous fibrosis are liable to spoil the cosmetic result.

The operations for ptosis, ectropion, and entropion are orthodox and sound. The book gives a good simple account of the principles and practice of reconstructive work on the eyelids, conjunctiva, and orbit. The book is well printed and produced, and the illustrations are clear.

NOTES

British Medical Bulletin

The *British Medical Bulletin*, vol. 9, No. 1, 1953, is devoted to the Physiology of Vision. It contains the following papers, abstracts of which will appear in *Ophthalmic Literature*.

Introduction.—Sir John Parsons.
Some problems concerning the formation and circulation of the aqueous humour—Hugh Davson.
Factors controlling ocular accommodation.—E. F. Fincham.
The nature of refractive errors.—Arnold Sorsby.
The interpretation of spectral sensitivity curves.—H. J. A. Dartnall.
Chromatic aberration and the polychromatic theory.—Hamilton Hartridge.
Defective colour vision.—W. D. Wright.
Visual properties studied by subjective measurements on the colour-adapted eye.—W. S. Stiles.
Stimulation of the retina with light fields of small size.—L. C. Thomson.
Colour vision in the peripheral retina.—R. A. Weale.
The absolute sensitivity of the eye and the variation of visual acuity with intensity.—M. H. Pirenne.
Electric records from the vertebrate optic nerve.—W. A. H. Rushton.
Physiology of vision: a bibliography of British books and periodicals.—Katharine Tansley.

Ophthalmological Society of the United Kingdom

At the XVII International Congress of Ophthalmology, New York, 1954, a new edition of the *Indicia* will be issued under the title *Index Ophthalmologicus*. The Ophthalmological Society has been asked to prepare the section covering Great Britain.

Any ophthalmologist, not already a member of the Ophthalmological Society of the United Kingdom or of the Faculty of Ophthalmologists, who wishes to be included in this Index, is asked to send his name and address to the Honorary Secretary, 45 Lincoln's Inn Fields, London, W.C.2., *before April 30, 1953.*

Ophthalmological Society of Mexico

At a meeting held on February 3, 1953, the following officers were appointed:

President: Dr. Manuel de Rivas Cherif *Perpetual Secretary:* Dr. Raul A. Chavira
Vice-President: Dr. Anselmo Fonte Barcena *Annual Secretary:* Dr. Emilio Remolina Lopez
Treasurer: Dr. Jorge Meyran

Contact Lens Society

The Annual General Meeting of the Contact Lens Society will be held on Monday *April 20, 1953, at 6.30 p.m.* at the Institute of Ophthalmology. It will be followed at approximately 7.15 p.m. by a clinical session in which Messrs. F. Burnett Hodd, F. Dickinson, F. Williamson-Noble, and Norman Bier will take part.

Mr. John Foster, F.R.C.S., senior clinical lecturer in ophthalmology at Leeds University, has been invited to lecture at the University of Barcelona, Spain, and at the Triennial Congress of Ophthalmology at Bueños Aires, Argentina, in April, 1953.

OBITUARY

Ransom Pickard

Ransom Pickard, C.B., C.M.G., M.D., M.S., Lond., F.R.C.S., D.P.H., who died on February 9, 1953, at the age of 85, graduated at Bart's in 1889, and after holding house appointments there and taking the higher qualifications went to Moorfields. He settled in Exeter in the early '90s, and carried on a general practice there. On the formation of the Territorial Army he was largely instrumental in raising the 1st Wessex Field Ambulance, and in 1914 he went to France with a regular division; he was soon appointed a C.M.G., and later became A.D.M.S. of the 48th division. For his work in Germany after the armistice he was made a C.B.

On his return to Exeter he began to specialize in ophthalmology. Having early learnt to regard his patients as an entity and not to pay sole regard to the narrow limits of the eye, he was ready to tackle any general operation, and aural surgery particularly interested him. He was appointed Surgeon to the West of England Eye Infirmary (founded the year after Moorfields), and served there with skill and devotion until his retirement. Always ready to learn, he was one of the first to undertake trephining, and when the slit lamp was introduced he went to Vienna to study its uses. Throughout his career he made an estimation and drawing of the size and depth of the cup in every patient, and this led him to investigate the subject of cavernous atrophy and its relation to glaucoma, on which he wrote several papers. His remarks in discussions at the meetings of the local Medico-Chirurgical Society showed his wide knowledge of general medicine and surgery. He was particularly proud of being elected President of the Ophthalmic Section of the Royal Society of Medicine, an honour which rarely went to a provincial surgeon.

Ransom Pickard made an active contribution to each phase of life in which he took part. He held every office open to a layman in the Methodist connexion; he was elected mayor of Exeter in 1927; and he was a Vice-President of the University College of the South-West. His leisure interests were equally numerous and enthusiastic. He was a member of the Alpine Club, a Vice-President of the Exeter Drama League, and Past-President of both the Devonshire Association and the Devon Archaeological and Exploration Society. He would take long walks on Saturdays whatever the weather, and return with his pockets full of specimens. To converse with him was to learn much from his deep and various knowledge. He leaves a widow who shared his many interests and was a great help and support to him in all of them.